DEFICIENCY AND DUPLICATIONS FOR THE GENE BOBBED IN DROSOPHILA MELANOGASTER

N. P. SIVERTZEV-DOBZHANSKY AND TH. DOBZHANSKY
California Institute of Technology, Pasadena, California

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INTRODUCTION

The present paper consists of two parts. The first deals with a description of a deficiency involving the locus of the sex-linked gene bobbed. The results obtained have a bearing on the problems of the cytological map of the X chromosome and on the existence of an "inert" region in this chromosome. The second part of the paper is devoted to the study of the interactions between bobbed-deficiency and various duplications covering parts of the region lost in the deficiency. The results may have a bearing on the problem of position effect.

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ORIGIN OF THE DEFICIENCY

Wild-type males from the "Oregon" stock were treated with a heavy dose of X-rays and crossed to females having attached X chromosomes ($\widehat{XX}$). In the offspring of this cross a single male was found (February 1931) which had slightly rough eyes, and short, parallel-sided, and somewhat truncate wings. This aberrant male was crossed to unrelated $\widehat{XX}$ females; all the male offspring exhibited the characteristics of the father. The mutant males were then crossed to wild-type females; the F_1 generation consisted of normal flies. In F_2 slightly less than one-half of the males showed the mutant characters. It was concluded that the new mutant is a sex-linked recessive. Further tests showed that the new gene is an allelomorph of the previously known sex-linked recessive small-wing (*sl*^{1}, located at 54.2 in the X chromosome, see MORGAN, BRIDGES, STURTEVANT, 1925). The new mutant is, therefore, called small-wing-2 (*sl*^{2}). The external effects of *sl*^{2} are similar, though perhaps slightly more extreme, than those of *sl*^{1}.

An attempt to establish a stock homozygous for *sl*^{2} failed, the chromosome carrying *sl*^{2} being lethal when present in two doses in females. This was unexpected since *sl*^{1} is equally viable in females and in males. Further tests showed, however, that the lethal effect of the chromosome carrying *sl*^{2} is not associated with the gene *sl*^{2} itself, but with another locus in the

same chromosome. The locus responsible for the lethal effect is that of the gene bobbed (*bb*, 70.0 in the X chromosome). The presence of a bobbed-allelomorph in the chromosome carrying sl^2 was ascertained by the following test. The sl^2 males were crossed to females homozygous for bobbed. The results are shown in table 1. Females carrying a sl^2 X chromosome and a *bb* X chromosome are extreme bobbed in appearance. In fact, they

TABLE 1

$sl^2 bb^{def}$ ♂ × *bb* ♀

EXTREME BOBBED ♀	WILD-TYPE ♀	EXTREME BOBBED ♂	WILD-TYPE ♂
46	3	2	138

are considerably more extreme than females homozygous for *bb*. It follows that the chromosome carrying sl^2 contains an allelomorph of *bb*. For reasons to be presented below this new allelomorph of *bb* is called bobbed-deficiency (bb^{def}). The X-ray treatment induced, therefore, two independent mutations in the same chromosome, namely sl^2 and bb^{def}. The lethal effect of the sl^2-bb^{def} chromosome is to be ascribed to bb^{def} rather than to sl^2. Indeed, lethal allelomorphs of *bb* are known (bobbed-lethal, bb^l, see MORGAN, STURTEVANT and BRIDGES 1927, STERN 1929a).

The non-appearance of the bobbed-characteristics (short bristles, late hatching from the pupae, sometimes also disarrangement of the abdominal tergites), as well as the viability of the bb^{def} males, is to be expected. STERN (1925, 1927, 1929a) has shown that bobbed is the only known sex-linked gene having a wild-type allelomorph in the Y chromosome. The effect of bb^{def} in the males is usually suppressed by the Y chromosome. Only in males having no Y chromosome (XO males), or in males having a bobbed-allelomorph in the Y chromosome, can the bobbed characters be manifested.

In the offspring of the cross shown in table 1 there were three wild-type females and two extreme bobbed males. These classes are due to non-disjunction of the X and Y chromosomes in the males carrying the sl^2–bb^{def} chromosome. Such males occasionally produce spermatozoa containing both the X and the Y chromosomes, and spermatozoa containing neither of these chromosomes. The first kind of spermatozoa gives rise to XXY females. In such females the effect of bb^{def} is suppressed by the wild-type allelomorph of *bb* in the Y chromosome, and such females are wild-type in appearance. The second kind of spermatozoa produces XO males which are extreme bobbed (XO males manifest bobbed in a more extreme form than females homozygous for bobbed, STERN 1927). Non-disjunction of the X-Y pair of chromosomes occasionally takes place in normal males (STERN 1929b), but its frequency is very low. The frequency observed in

our experiment (2.6 percent, table 1) is higher than normal. It seems that the presence of the sl^2–bb^{def} chromosome increases the frequency of non-disjunction in males.

REDUCTION OF CROSSING OVER PRODUCED BY THE sl^2–bb^{def} CHROMOSOME

Small-wing-2 males were crossed to females homozygous for the combination of the sex-linked recessives known as "X-ple" (*sc ec cv* ct^6 *v* g^2 *f*). The F_1 females were crossed to white males (white is a sex-linked recessive). The results are presented in table 2. The calculated frequencies of crossing over are shown in table 3. The standard frequencies of crossing over in the X chromosome are given in table 3 for comparison (according to BRIDGES and OLBRYCHT 1926, and ANDERSON 1929).

TABLE 2

sc ec cv ct v g 6 7 f
1 2 3 4 5 *sl* *bb-def* ♀ × *w*♂.

MALES ONLY			
0—*sc ec cv ct v g f*	927	6—*f*	1
0—*sl*	1280	7—*sc ec cv ct v g*	1
1—*sc sl*	5	7—*sl f*	1
1—*ec cv ct v g f*	9	3, 6—*sc ec cv f*	1
2—*sc ec sl*	3	3, 7—*ct v g*	1
2—*cv ct v g f*	2	4, 5—*v sl*	1
3—*sc ec cv sl*	2	4, 6—*sc ec cv ct f*	4
3—*ct v g f*	3	4, 6—*v g sl*	1
4—*sc ec cv ct sl*	6	5, 6—*g sl*	1
4—*v g f*	11	5, 7—*g*	1
5—*sc ec cv ct v sl*	7	non-disjunction—*w*	46
5—*g f*	6	Total	2320

TABLE 3

Frequency of crossing over in females heterozygous for the X chromosome carrying bb^{def}.

INTERVALS	sc-ec	ec-cv	cv-ct^6	ct^6-v	v-g^2	g^2-sl^2	sl^2-f
bb^{def}	0.6	0.2	0.3	1.0	0.7	0.4	0.2
Standard	6.8	9.7	8.4	14.8	11.2	11.3	
Difference	−6.2	−9.5	−8.1	−13.8	−10.5	−10.7	

The very strong reduction of the frequency of crossing over in the chromosome carrying sl^2 and bb^{def} is obvious. The following experiments were undertaken in order to determine the frequency of crossing over to the right of the locus of *f*; the intervals lying to the right of *f* were not followed in the experiment described above. Small-wing-2 bb^{def} males were crossed

to carnation females (the gene carnation, *cr*, lies at about 8 units to the right of *f*), and the F_1 females were outcrossed to *w* males. Table 4 shows the results. In the presence of bb^{def} the frequency of crossing over between

TABLE 4

$\frac{sl^2 \quad bb^{def}}{cr}$ ♀ × *w*♂ (males only).

sl	*cr*	sl^2 *cr*	WILD-TYPE	*w*	TOTAL
849	1282	12	6	35	2184

sl^2 and *cr* is 0.9 percent instead of about 10 percent. In another experiment sl^2 bb^{def} males were crossed to *cr* females, and the F_1 females were outcrossed to *bb* Y^{bb} males (Y^{bb} is an allelomorph of *bb* lying in the Y chromosome; males having *bb* in the X and Y^{bb} in the Y chromosome manifest the characters of bobbed). The results are shown in table 5. The frequencies of crossing over in the sl^2–*cr* and the *cr*–bb^{def} intervals are 0.5 percent and 0.9 percent respectively. Since *sc* and *bb* are located in opposite ends of the X chromosome, the data presented in tables 2 to 5 show that crossing over is strongly reduced throughout the entire X chromosome.

TABLE 5

$\frac{sl^2 \quad bb^{def}}{cr}$ ♀ × *bb* Y^{bb}♂ (*males only*).

sl^2 *bb*	*cr*	sl^2 *cr*	*bb*	sl^2	*cr bb*	EXTREME-*bb*	TOTAL
1385	1861	9	8	20	11	36	3330

A considerable number of flies due to non-disjunction of the X and Y chromosomes appear in the offspring of females heterozygous for sl^2 and bb^{def} (*w* flies in tables 2 and 4, extreme-bobbed flies in table 5). The frequency of non-disjunctional flies varies from 1.1 percent to 2 percent in the different experiments. The normal frequency of non-disjunctional gametes is about 1:1200 (MORGAN, BRIDGES, STURTEVANT 1925). It is concluded that the sl^2–bb^{def} chromosome is responsible not only for the reduction of crossing over but also for the increase in the frequency of non-disjunction.

SEPARATION OF BOBBED-DEFICIENCY FROM SMALL-WING-2

Since crossing over occurs between sl^2 and bb^{def}, these genes can be separated from each other. The sl^2 *f* male shown in table 2, and the sl^2 *cr* males shown in table 4, carry the left part of the original sl^2–bb^{def} chromosome but, presumably, do not carry its right part (containing bb^{def}). Conversely, the wild-type males shown in table 4 carry the right part of the

original chromosome but do not carry its left part (containing sl^2). Homozygous sl^2 f and sl^2 cr stocks were established without difficulty. An attempt to establish a stock homozygous for bb^{def}, but not carrying sl^2, failed. The lethal effect of the sl^2–bb^{def} chromosome in double dose in females is clearly due to bb^{def} and not to sl^2.

The following experiment shows that sl^2 without bb^{def} does not influence the frequency of crossing over in the X chromosome. Crosses were made of sl^2 cr males to y cv v f females. The F_1 females were crossed to white-eyed males. Among the 1573 males counted in the next generation there were only two white ones; the frequency of non-disjunction in the presence of sl^2, without bb^{def}, is not significantly different from normal (see above). Table 6 shows the frequency of crossing over observed in this experiment. The frequencies observed are not significantly different from the standard values.

TABLE 6

$\frac{y\ cv\ v\ \ f}{sl\ \ cr}$ ♀ × w♂ (*males only*).

INTERVAL	y–cv	cv–v	v–sl	sl–f	f–cr
Frequency	12.0	20.7	19.6	2.4	8.2

Males carrying bb^{def} without sl^2, as well as females heterozygous for bb^{def}, do not differ from the wild-type in appearance, but their viability and fertility seem to be somewhat below that of the wild-type. The presence of bb^{def} may be detected phenotypically by making flies heterozygous for other allelomorphs of bb. Thus, females of the structure bb/bb^{def} are extreme bobbed (table 1). Likewise, males of the structure bb^{def}/Y^{bb} show the bobbed characteristics in a more extreme form than males of the constitution bb^{t}/Y^{bb}, but much less extreme than XO males carrying bb. The allelomorph bb^{def} seems, therefore, to be completely recessive to the wild-type allelomorphs located in either X or in Y chromosomes; in compounds with other bb allelomorphs it behaves as the most extreme allelomorph of bb thus far known.

PROBABLE NATURE OF bb^{def}

Point-mutations do not, as a rule, affect the frequency of either crossing over or non-disjunction of the chromosomes in which they lie. On the contrary, chromosomal aberrations, such as translocations, inversions, deficiencies, and duplications, frequently affect both crossing over and disjunction. The behavior of bb^{def} suggests, therefore, that not a point-mutation, or at least not only a point-mutation, but some chromosome-aberration is responsible for its appearance and the manner of its action. Tests

were made for the presence of a translocation associated with bb^{def}. The results were clearly negative for all the chromosomes. The assumption that an inversion arose in the X chromosome simultaneously with bb^{def} is improbable, since the presence of bb^{def} allows some single crossing over to take place in every interval studied; this is not the case in any known inversions.

The mutation from wild-type to bb^{def} is best interpreted as due to a deficiency, that is, to the loss of a section of the X chromosome carrying the normal allelomorph of bobbed. According to MOHR (1923, 1927, 1929) deficiencies behave as the most extreme allelomorphs of the known recessives whose loci are included in the deficient region. This is the case with bb^{def} (see above). Like all the other known deficiencies bb^{def} is lethal when homozygous. All known deficiencies eliminate crossing over in the sections

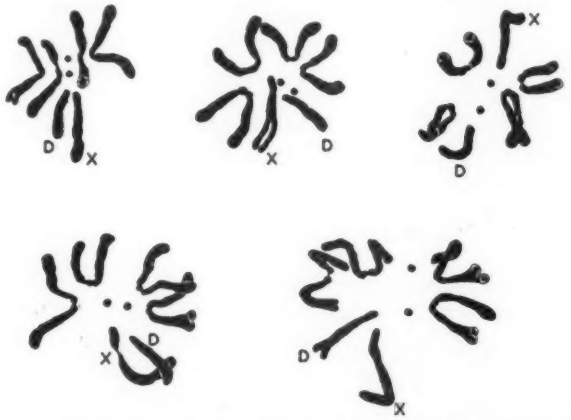

FIGURE 1.—Chromosomes of females heterozygous for bobbed-deficiency. X, the normal X chromosome; D, X chromosomes carrying the deficiency.

involved (BRIDGES 1917, MOHR 1923, 1927), but the longest ones also decrease the frequency of crossing over in the sections of the chromosome adjacent to the deficient section (LI and BRIDGES 1929, MORGAN, BRIDGES and SCHULTZ 1931). The cytological findings (see below) prove that bb^{def} is actually a deficiency.

CYTOLOGY OF BOBBED-DEFICIENCY

The phenomenon of deficiency was discovered and studied in Drosophila by BRIDGES (1917, 1919) and by MOHR (1919, 1923, 1927, 1929). BRIDGES (1917) defined the term "deficiency" to mean "the loss or inactivation of an entire, definite, and measurable section of genes and framework of a chromosome." The deficiencies studied by BRIDGES and MOHR were short

and invisible cytologically. The assumption that the deficient genes were actually lost from the chromosome was inferred from the genetic data. For this reason BRIDGES admitted the possibility that the deficient genes were not physically lost but merely inactivated.

Some of the more recently described deficiencies have proved to be sufficiently extensive to produce cytologically visible changes in the chromosomes. To this group belong the "v–o" deficiency in mice (PAINTER 1927), several deficiencies in the X chromosome of Drosophila (L. V. MORGAN in MORGAN, BRIDGES and STURTEVANT 1928, PAINTER and MULLER 1929, MULLER and PAINTER 1932, DOBZHANSKY 1932a, b), and in *Zea mays* (MCCLINTOCK 1931). PAINTER (1927) proposed the term "deletion" for cases in which the loss of genes is discoverable both genetically and cytologically. Evidently, most if not all of the deficiencies studied by BRIDGES and MOHR are deletions in PAINTER's sense. The existence of two terms for precisely the same phenomenon seems unnecessary.

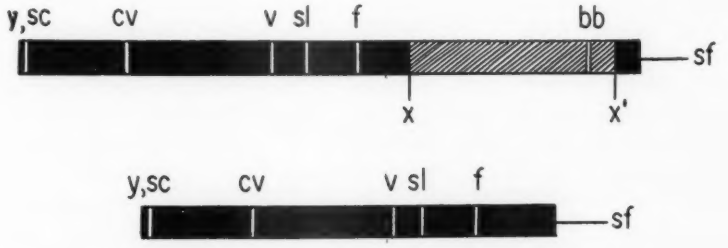

FIGURE 2.—The normal X chromosome (above); and the X chromosome carrying bobbed-deficiency (below). X and X^1, the points at which the chromosome was broken. The shaded part, the section which is lost in the deficiency. *sf*, the spindle fibre. Other letters, location of various genes.

Bobbed-deficiency behaves genetically like the deficiencies studied by BRIDGES and MOHR. The deficiency-nature of the allelomorph bb^{def} was inferred from the genetic data presented above. This deficiency involves the locus of a single known gene, namely that of bobbed. Nevertheless, it is clearly visible cytologically.

Wild-type females were crossed to $sl^2\ bb^{def}$ males. All the females in the offspring of this cross should be heterozygous for bb^{def} (barring the possibility of primary non-disjunction). Female larvae were selected, and their nerve-ganglia were fixed in Navashin's solution. Several chromosome-plates were found in this material. Some of them are reproduced in figure 1. In each plate one may see two X chromosomes which are unequal in length. The longer chromosome (X) is presumably the normal X chromosome. The shorter one (D) is the X chromosome deficient for bobbed. The

shorter chromosome is approximately two-thirds the length of the longer chromosome present in the same plate. Hence, in bobbed-deficiency, about one-third of the X chromosome is lost.

Figure 2 represents schematically the structure of the X chromosome in bobbed-deficiency, and also an interpretation of the mode of its origin. The normal X chromosome (figure 2A) was broken in two places (x and x^1), and the section lying between x and x^1 was lost. The sections lying to the left and to the right of x and x^1 respectively became united, producing the bobbed-deficiency chromosome (figure 2B). An alternative assumption is that the X chromosome was broken at a single locus (that is, at x), and that the section lying to the right of x was lost. Such an assumption seems, however, improbable since the spindle-fibre is known to be attached to the right end of the X chromosome. The behavior of translocations and deficiencies in Drosophila indicates that a chromosome which has lost its spindle-fibre attachment does not behave normally in mitosis, and is therefore eliminated.

DUPLICATIONS FOR BOBBED

Several duplications for various sections of the X chromosome were found in the progeny of males treated with X-rays. The origin of these duplications is mostly due to a loss ("deletion," PAINTER and MULLER, 1929) of the middle region of the chromosome, followed by a reunion of the end regions. The resulting chromosomes contain, consequently, only a part of the genes normally located in the X, and consist of two parts corresponding to the left and the right ends of the normal X respectively. Most of the duplications are so small that their addition to the chromosomal compliment of males (respectively, females) does not upset the sex balance of the resulting hyperploid individuals. Thus, a duplication female carries two normal X's plus the duplication, that is, a fragment of a third X. Similarly, a duplication male carries one normal X, one Y chromosome, and the duplication.

Six different duplications, covering small portions of the left end of the X chromosome, were selected for the purposes of the present study. They are denoted as "Duplication 101" (described in DOBZHANSKY 1932a), "Duplication 106," 107, 118, 136 (DOBZHANSKY 1932b), and "Duplication 135" (undescribed). The methods used for determination of the loci present in the duplications were outlined in the papers just referred to, and are, therefore, only briefly reviewed here. Individuals are obtained which are homozygous for definite recessive sex-linked genes, and which carry the duplication in question. If the duplication contains the dominant wild-type allelomorphs of the respective recessives, the duplication-carrying individuals fail to manifest the characteristics of the recessives (the duplications contain only wild-type allelomorphs of the sex-linked recessives since

the individuals used for X-rays treatment are usually wild-type males). It was found by this method that duplications 101, 106, 107, 118, and 135 carry the loci of yellow (*y*), scute (*sc*) and silver (*sv*). The duplication 136 carries in addition the loci of kurz (*kz*), broad (*br*) and prune (*pn*).

For the determination of the presence of the locus for bobbed, a slightly different method is used. Females having attached X chromosomes ($\widehat{XX}$), and homozygous for certain sex-linked recessives (the genetic structure of such females is represented in the upper left corner of figure 3), are crossed to bb/Y^{bb} males (upper right corner of figure 3; Y^{bb} is an allelo-

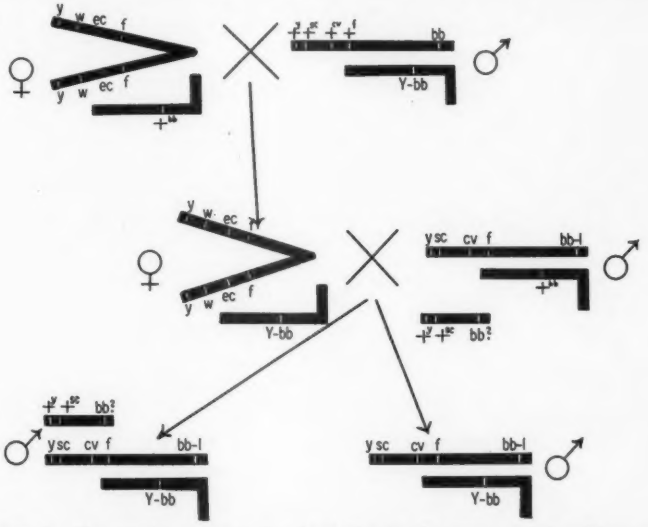

FIGURE 3.—The experimental procedure for testing for the presence of the wild-type allelomorph of bobbed in the duplications. The >shaped chromosome, the attached X chromosomes ($\widehat{XX}$); the rod-shaped chromosome, the X chromosome; the hook-shaped chromosome, the Y chromosome; the short rod-shaped chromosome, the duplication.

morph of bobbed located in the Y chromosome). Females produced in the offspring of this cross are $\widehat{XX}/Y^{bb}$ (figure 3). Such females are crossed to males carrying the recessives *y*, *sc*, *cv*, *f* and bb^l in their normal X's, and carrying the duplication to be tested. In the next generation two kinds of males are obtained (lower line in figure 3). Both kinds carry bb^l and Y^{bb}, but one kind carries the duplication, and the other is free from it. If the duplication does not contain the locus of bobbed, all males show bobbed in a rather extreme form. If the wild-type allelomorph of bobbed is present in the duplication, the resulting male is non-bobbed. The presence of duplications in a given male is recognized by the suppression of the effects of *y* and *sc*. The results of testing the duplications for the presence of the

locus for bobbed are shown in table 7. One may conclude that duplication 106 does not carry the locus of bobbed, while all other duplications do carry that locus.

TABLE 7

$y^2 w^a$ ec f $\widehat{XX}$ / Y^{bb} ♀ × y sc cv f bb^1 / Y / duplication ♂.

DUPLICATION	$y^2 w^a$ ec f ♀	w^a ec f ♀	y sc cv f bb ♂	cv f bb ♂	cv f ♂
101	274	349	326	..	241
106	190	316	205	67	..
107	96	222	165	..	128
118	115	259	150	..	116
135*	161	314	168	..	134
136*	75	146	81	..	59

* Duplications 135 and 136 carry y^2 (an allelomorph of y) in the fragment. For testing them $\widehat{XX}$ yellow females were used instead of the $\widehat{XX}$ y^2 w^a ec f ones.

A different method of testing for the presence of the bobbed-locus in the duplications consists in using females of the structure bb/bb^1. Such females are, in the absence of duplications, extreme bobbed in appearance (figure 4). If a duplication containing the wild-type allelomorph of *bb* is added to the chromosomes of such females (the resulting constitution is bb/bb^1/duplication, figure 4), the flies are wild-type. The duplications were also tested by this method; the results were the same as those presented above.

It should be kept in mind, that both methods of testing for the presence of the locus for bobbed in duplications have the following limitation. It is assumed that the duplications may carry either the wild-type allelomorph of bobbed, or not carry the bobbed locus at all. This assumption is based on the fact that the duplications are obtained by treating wild-type (that is, non-bobbed) males with X-rays. However, the X-ray treatment may induce a mutation to bobbed in the duplicating fragment itself. It is easy to see that in such a case the tests may lead to the conclusion that the duplications do not carry bobbed at all. The negative results of the tests are thus not entirely conclusive, while the positive results are more convincing.

INTERACTION OF BOBBED-DEFICIENCY WITH BOBBED-DUPLICATIONS

Bobbed-deficiency behaves as a recessive. Its external effects are suppressed by the presence of a single wild-type allelomorph of bobbed. Thus, females of the constitution $bb^{def}/+^{bb}$, and males of the constitution bb^{def}/Y^+ (figures 4 and 5), are wild-type. Five of the six duplications described above seem to carry wild-type allelomorphs of bobbed. It is, consequently, justifiable to expect that the presence of these duplications will

suppress the effects of bobbed-deficiency. The experiments show that this expectation is not realised.

Males of the constitution bb^{def}/Y^{bb}/duplication were secured. The experiments were so arranged, that males of the constitution bb^{def}/Y^{bb} were obtained in the same cultures (the experimental procedure applied for getting the bb^{def}/Y^{bb}/duplication and the bb^{def}/Y^{bb} males is similar to that diagrammed in figure 3. The difference consists in using *y sl*^{2} bb^{def}/Y^+ males instead of the *y sc cv f* bb^1 males shown in figure 3). The two kinds of males are exactly alike, except for the presence of the duplication. The inspection of the bb^{def}/y^{bb}/duplication males has shown that they are not wild-type, as expected, but are more or less extreme bobbed. Their bristles are short and slender, the abdominal tergites are frequently disarranged, the viability is low. In the absence of bobbed-deficiency the duplications do not produce these characteristics. It seems clear that the wild-type allelomorph of bobbed lying in the duplications fails to suppress bobbed-deficiency.

Some measurements were undertaken for the purpose of obtaining more precise information on the degree of suppression of bb^{def} by various duplications. Bristles were measured in flies of the constitution bb^{def}/Y^{bb}/duplication and bb^{def}/Y^{bb}. Flies were macerated in a solution of KOH. After washing in water, the flies were transferred in glycerine for clearing. The heads and the thoracal parts of the cleared flies were then isolated, arranged in rows in drops of glycerine on slides, covered with thick cover slips, and flattened as much as possible by means of pressing on the cover slips. The length of the inner vertical, the posterior dorsocentral, and the posterior scutellar bristles was then measured in terms of the units of an eyepiece-micrometer (1 unit being equal to 8.9 mikra). Only one bristle of each kind was measured in each fly. By the "length of the bristle" is meant the distance between the free end of the bristle and its insertion into the theca, irrespective of whether the bristle is absolutely straight or slightly curved.

The results of the measurements are presented in tables 8, 9, and 10. The graphs marked "duplication" indicate the length of the bristles in bb^{def}/Y^{bb}/duplication flies; the graphs marked "control" give similar data for the bb^{def}/Y^{bb} flies from the same cultures. The data for the bb^{def}/Y^+ (wild-type) flies serve as the standard of comparison. For each of the forms studied the mean value (M), its mean error (m), the standard deviation (σ), coefficient of variation (C), the limits of variation (Lim), and the number of flies measured (n) are given.

The bristles in the bb^{def}/Y^{bb}/duplication males are in no case as long as they are in wild-type (bb^{def}/Y^+) males. The differences are statistically significant, with a single exception of the dorsocentral bristles in duplica-

TABLE 8.

Length of the inner vertical bristle in bb^{def}/Y^{bb}/ duplication and in bb^{def}/Y^{bb} males.

	$M \pm m$	$\sigma = \pm$	C	*Lim*	*n*
bb^{def}/Y^{+} (wild-type)	30.87±0.20	1.78	5.8	25–35	81
Duplication 101	24.71±0.22	1.79	7.3	21–35	68
Control 101	20.00±0.40	1.94	9.7	16–24	24
Duplication 106	21.06±0.32	2.36	11.2	16–25	54
Control 106	20.67±0.57	2.82	13.6	17–28	24
Duplication 107	27.29±0.22	1.98	7.3	22–31	81
Control 107	21.14±0.50	2.52	11.8	17–25	25
Duplication 118	21.48±0.27	1.72	8.0	18–27	41
Control 118	21.14±0.45	2.24	10.6	15–24	25
Duplication 135	27.62±0.43	1.74	6.3	25–30	16
Control 135	23.03±0.35	1.36	5.9	21–25	15
Duplication 136	20.68±0.30	1.83	8.9	18–25	37

TABLE 9

Length of the posterior dorsocentral bristle in bb^{def}/Y^{bb}/ duplication and in bb^{def}/Y^{bb} males.

	$M \pm m$	$\sigma = \pm$	C	*Lim*	*n*
bb^{def}/Y^{+} (wild-type)	36.84±0.25	2.30	6.3	30–40	81
Duplication 101	32.26±0.26	2.13	6.6	26–38	68
Control 101	25.83±0.47	2.29	8.9	22–30	24
Duplication 106	27.91±0.40	2.94	10.5	23–33	54
Control 106	27.25±0.74	3.60	13.2	23–36	24
Duplication 107	34.62±0.24	2.18	6.3	30–40	81
Control 107	28.18±0.39	1.94	6.9	24–32	25
Duplication 118	28.02±0.31	1.96	7.0	24–32	41
Control 118	28.18±0.42	2.08	7.4	22–30	25
Duplication 135	35.75±0.39	1.56	4.4	32–38	16
Control 135	29.43±0.37	1.42	4.8	27–32	15
Duplication 136	26.68±0.23	1.40	5.2	24–30	37

TABLE 10

Length of the posterior scutellar bristle in bb^{def}/Y^{bb}/duplication and in bb^{def}/Y^{bb} males.

	$M \pm m$	$\sigma = \pm$	C	*Lim*	*n*
bb^{def}/Y^{+} (wild-type)	47.91±0.22	1.95	4.1	43–52	81
Duplication 101	38.62±0.21	1.71	4.4	35–42	68
Control 101	33.42±0.67	3.31	9.9	29–40	24
Duplication 106	33.87±0.42	3.06	9.0	28–39	54
Control 106	33.92±0.98	4.80	14.2	27–42	24
Duplication 107	43.34±0.23	2.10	4.8	38–48	81
Control 107	35.62±0.80	4.02	11.3	27–44	25
Duplication 118	35.08±0.34	2.18	6.2	30–40	41
Control 118	35.70±0.51	2.54	7.1	27–38	25
Duplication 135	44.88±0.44	1.76	3.9	42–49	16
Control 135	36.23±0.42	1.76	4.9	34–48	15
Duplication 136	33.00±0.31	1.86	5.6	30–37	37

tion 135. It follows that none of the duplications studied suppress completely the effects of bobbed-deficiency. On the other hand, the degree of suppression produced by different duplications is variable. Duplication 135 comes closest to producing a complete suppression. Duplication 107 follows next. Duplication 101 has, in the presence of bb^{def}, bristles roughly intermediate in length between those observed in wild-type and in bb^{def}/Y^{bb} males. Finally, duplications 118 and 136 behave very nearly as duplication 106, in spite of the fact that the two former carry the wild-type allelomorph of bobbed, and the latter has no bobbed locus at all.

The effect of duplications on bb^{def} was also tested by a different method. Females of the constitution *ClB*/*y* sl^2 bb^{def} were crossed to *y* bb/Y^+/duplication males. Some of the non-bar females obtained in the next generation were yellow, and others were non-yellow. The yellow females have the constitution *y* sl^2 bb^{def}/bb, and the non-yellow ones the constitution *y* sl^2 bb^{def}/bb/duplication. If the duplications suppress the effects of bb^{def}, the *y* sl^2 bb^{def}/bb/duplication females should be non-bobbed. In fact they are more or less distinctly bobbed. The behavior of the different duplications in females is similar to their behavior in males. Thus, duplications 107 and 135 produce a fairly strong, though incomplete, suppression of bb^{def} in females, while duplication 118 fails to produce marked effects.

The facts presented above apparently leave no escape from the conclusion that the behavior of the wild-type allelomorph of bobbed lying in duplicating fragments is different from the behavior of the same allelomorph lying in the complete X or Y chromosomes. It is worth while to consider here some sources of error, which might conceivably invalidate the above conclusion. One may suppose, for instance, that the presence of the duplications, by altering the genic balance, is *per se* responsible for a decrease (or an increase) of the length of the bristles. This supposition is invalid, since in the absence of bobbed, the duplications (perhaps with the single exception of duplication 136, which makes the bristles thicker and shorter), do not seem to affect the size of the bristles. It is, of course, possible that a very slight effect on the bristle size is produced by the duplications. It remains, however, to be explained why different duplications behave so differently in compounds with bb^{l} on one hand and in compounds with bb^{def} on the other.

One may also suppose that the allelomorph Y^+ (that is, the wild-type allelomorph of bobbed lying in the Y chromosome) is more effective in suppressing bb^{def} in males than is $+^{bb}$ (that is, the wild-type allelomorph of bobbed lying in the X chromosome). If this were so, then the difference in the bristle length which exists between the bb^{def}/Y^+ and the bb^{def}/Y^{bb}/duplication males might be attributed to the higher efficiency of Y^+ as compared with $+^{bb}$. This supposition is contrary to the following two

facts: (a) different duplications behave differently in the compound bb^{def}/Y^{bb}/duplication; (b) the behavior of the duplications is similar in both sexes.

DISCUSSION OF THE RESULTS

The inert region of the X chromosome

Bobbed-deficiency represents a loss of about one-third of the whole X chromosome. A loss of such a long section of a chromosome might be expected to produce a considerable upset of the genic balance, and, consequently, a somatic effect. This is not the case with bobbed-deficiency. As shown above, bb^{def} is lethal when homozygous, but has little, if any, effect on the heterozygotes. Females of the constitution $bb^{def}/+$, and males

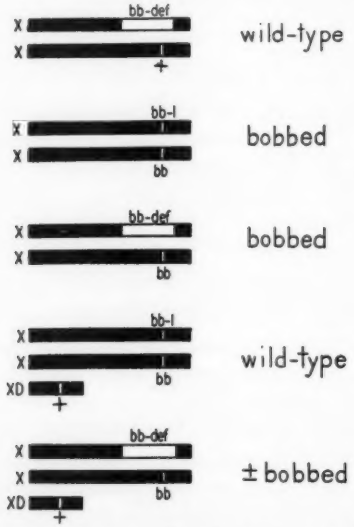

FIGURE 4.—The interaction of the different bobbed allelomorphs in females, X, the X chromosome; XD, the duplication; the white area, the section which is lost in bobbed-deficiency.

of the constitution bb^{def}/Y^{+} have a somewhat lower viability than wild-type flies, but they are seemingly completely normal in appearance. Deficiencies which are too short to be visible cytologically frequently produce striking dominant somatic effects (MOHR 1923, 1929), and relatively very short deficiencies may be completely lethal in heterozygotes (see, for example, DOBZHANSKY 1930 and 1931). This indicates that the region which is lost in bb^{def} is less important for development than regions of similar lengths in other chromosomes of Drosophila.

PAINTER (1931a, b), MULLER and PAINTER (1932), and DOBZHANSKY (1932b) have shown by combined genetical and cytological studies that

the right one-half or one-third of the X chromosome is made up of a region in which only one gene, namely bobbed, is known to be located. Furthermore, very little, if any, crossing over takes place in this region. PAINTER called this region the "inert region," implying that the number of functional genes per unit of distance is very small in this region. The behavior of bobbed-deficiency constitutes new and fairly conclusive evidence in favor of this view.

The material contained in the inert region of the X chromosome is supposed to be homologous to a section of the Y chromosome. The Y chromosome is also known to be composed of predominantly inert material. As a matter of fact, this assumption seems to have been warranted on the basis of facts which were discovered before anything was known regarding the

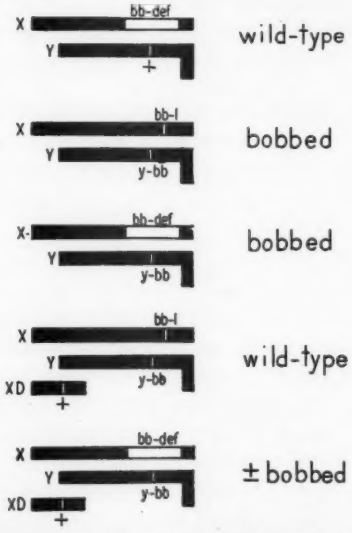

FIGURE 5.—The interaction of the different bobbed allelomorphs in males. Y, the Y chromosome. The significance of other letters is the same as in figure 4.

existence of an inert region in the X chromosome. METZ (1926) in his studies on the spermatogenesis of Drosophila has shown that the X and the Y chromosomes do not pair at synaptic stages along their entire lengths, but that only a part of the X undergoes pairing with the Y, the rest of the X remaining unpaired. This indicates that a part of the X is homologous with a part of the Y chromosome.

The behavior of bobbed-deficiency provides further confirmation of the above assumption. Bobbed-deficiency males are normal phenotypically. If some of the genes located in the region of the X which is lost in

bb^{def} have no allelomorphs in the Y chromosome, then bb^{def} males should not carry these genes at all. It is known, however, that all the known deficiencies so far studied in Drosophila are invariably lethal when homozygous. The data of LI (1926) show, furthermore, that individuals homozygous for deficiencies die in very early stages of embryonic development. These data, in the opinion of the present writers, indicate that most, if not all of the genes in Drosophila are essential for development, irrespective of whether the known mutations of these genes produce alterations of "superficial" or "fundamental" characters. From this point of view it is improbable that a male which has lost X chromosome genes having no allelomorphs in the Y chromosome would be at all viable, not to speak of being phenotypically normal.

The cytological findings in bobbed-deficiency serve to establish the minimum length of the inert region of the X chromosome. The length of this region is not less than one-third of the length of the X.

Behavior of the wild-type allelomorph of bobbed in duplications

Bobbed-deficiency is recessive to the wild-type allelomorph of bobbed, provided the latter lies in a normal, unbroken X or Y chromosome. Thus $bb^{def}/+^{bb}$ females and bb^{def}/Y^{+} males are wild-type. The situation is different if the wild-type allelomorph of bobbed lies in a fragment of an X chromosome. Five duplications carrying the locus of bobbed were studied. According to their origin they should carry the wild-type allelomorph of bobbed. None of these duplications suppresses the effects of bb^{def}. Individuals of the constitution bb^{def}/Y^{bb}/duplication and bb^{def}/bb/duplication are more or less clearly bobbed. Three possible explanations of this phenomenon may be discussed here.

First explanation

The observed facts may indicate that: (a) the development of the wild-type characteristics depends upon the presence of a definite amount of the substance located in the so-called "inert" region of the X, and in the corresponding region of the Y chromosome, rather than upon the action of a specific locus located in these regions; (b) the known allelomorphs of bobbed (bb^{def}, bb^{l}, bb, Y^{bb}) represent losses of varying amount of this substance, which, for the purposes of the present discussion we may call "the bobbed-substance"; (c) the smaller the amount of the "bobbed-substance" present in the germ plasm, the more extreme become the characteristics of bobbed in the adult fly.

None of the duplications studied include the entire inert region of the X chromosome (DOBZHANSKY 1932b). Hence, none of them carries the

amount of the "bobbed-substance" present in the normal X. The duplications include, however, enough of the "bobbed-substance" to produce the wild-type condition in the combinations bb^l/Y^{bb}/duplication and bb^l/bb/duplication. Since bb^{def} represents a longer deficiency than bb^l, the presence of the duplications is not sufficient to produce the wild-type condition in bb^{def}/Y^{bb}/duplication and bb^{def}/bb/duplication flies (figures 4 and 5).

STERN (1929a) has shown that accumulation of the bobbed allelomorphs in the germ plasm results in a gradual approach toward the wild-type condition in the phenotype. Thus, according to STERN, individuals of the constitution bb^l/bb^l are inviable, bb^l/bb are extreme bobbed, $bb^l/bb^l/Y^{bb}$ are less extreme, $bb^l/bb^l/Y^{bb}/Y^{bb}$ are still less extreme, $bb^l/bb/Y^{bb}$ are close to wild-type, and $bb^l/bb/Y^{bb}/Y^{bb}$ are not distinguishable from wild-type. It is easy to see that these results of STERN harmonize perfectly with the interpretation that all bobbed allelomorphs are deficiencies.

STERN discovered, however, another fact which is contradictory to our first explanation. The extreme bobbed allelomorph, known as bobbed-lethal (bb^l), was repeatedly observed to revert to a less extreme allelomorph (bb), and directly to wild-type. This fact is difficult to reconcile with the assumption that bb^l is a deficiency.

Second explanation

The wild-type allelomorph of bobbed is one of the frequently mutating loci. Spontaneous mutations from wild-type to various bobbed allelomorphs are rather common. Though the frequency of mutations at the bobbed locus under the influence of X-rays is unknown, it is not unreasonable to suppose that this frequency is high. The origin of the duplications is due to the breakages caused by X-ray treatment. It is, then, possible that mutations from wild-type to weak allelomorphs of bobbed were induced in the duplications at the time of their origin. This would explain the behavior of the duplications in combinations with bb^{def}.

This explanation meets with a difficulty, for every one of the five duplications studied needs to be supposed to carry a bobbed allelomorph induced by X-rays. Even if the mutation rate of the bobbed locus is much higher than that for any other known gene, it is very improbable that five duplications would by chance carry such mutations. If the phenomena observed are to be accounted for by mutations at the bobbed locus, an additional assumption is necessary, namely that the occurrence of a breakage in the chromosome strongly increases the probability of mutations taking place in the same chromosome. Such a possibility is, of course, not to be disregarded on *a priori* grounds. There exist, indeed, some facts which argue in favor of such possibility. Translocations very frequently carry lethals,

or mutations producing visible effects, the loci of which are associated with the loci at which the chromosomes were broken (MULLER and ALTENBURG 1930, DOBZHANSKY 1930, 1932c). STERN and OGURA (1931) observed mutations at the bobbed locus which arose simultaneously with translocations involving the X and the Y chromosomes.

Third explanation

The effect of the wild-type allelomorph of bobbed on development may depend upon its structure as well as upon its position in the chromosome. The loss of the middle part of the X chromosome involves a removal of the material normally located in the vicinity of bobbed, and establishing an association between the locus of bobbed and other loci lying normally far from bobbed. The behavior of the wild-type allelomorph of bobbed in the duplications may, thus, be accounted for by "position effect."

The phenomenon of position effect was discovered in Drosophila by STURTEVANT (1925, 1928), who demonstrated that two Bar genes lying in the same chromosome produce a stronger effect than two Bar genes lying in opposite chromosomes. The appearance of "mutations" at the loci of breakages in translocations (see above) may be accounted for by position effect as well as by mutation, and in some cases the explanation by position effect is distinctly preferable to that by mutation (DOBZHANSKY 1932c). The behavior of certain other genes, besides bobbed, in duplications also suggests the existence of a position effect (DOBZHANSKY and STURTEVANT 1932).

The second and the third explanations account equally well for the observed behavior of the wild-type allelomorph of bobbed in the duplications. At the present there seems to be no way for distinguishing between these two explanations experimentally. It is perhaps desirable to point out here that these two explanations may not be mutually exclusive. Mutation represents an alteration of the structure of the gene, and may arise without a breakage taking place in the vicinity of that gene. In case of mutation the alteration is permanent, in the sense that the original condition may be regained only by a reverse mutation. In case of position effect the alteration of the functioning of the gene is due to the removal of other genes normally lying in the neighborhood of the gene in question, or to the association with genes lying normally far from it. Hence, position effect should disappear as soon as the normal order of genes is restored. It is possible, however, that the bonds existing between the adjacent genes in the chromosome are so intimate, that the rupture of these bonds may lead to irreversible alterations in the structure of the gene itself. In such a way a permanent alteration of the structure of the gene (mutation) may be brought about by a change in the position of that gene in the chromosome (position effect).

SUMMARY

1. A deficiency for the sex-linked gene bobbed was found in the progeny of males treated with X-rays. The presence of the deficiency (symbol bb^{def}) decreases the frequency of crossing over, and increases the frequency of non-disjunction of the X chromosomes.

2. The X chromosome carrying the deficiency is about two-thirds as long as the normal X. It follows that the "inert" region makes up at least one-third of the length of the normal X.

3. The deficiency behaves as a recessive to the wild-type allelomorph of bobbed. Thus, $bb^{def}/+^{bb}$ females and bb^{def}/Y^{+} males are wild-type in appearance. In compounds with other allelomorphs of bobbed (bb, Y^{bb}) the deficiency produces an exaggeration of the bobbed characteristics.

4. Five duplications for sections of the X chromosome carrying the locus of bobbed are described. According to their origin these duplications should carry wild-type allelomorphs of bobbed.

5. The wild-type allelomorph of bobbed lying in the duplications fails to suppress the effects of bobbed-deficiency. Three possible explanations of this phenomenon are discussed.

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COMPARATIVE CYTO-GENETIC STUDIES OF TETRAPLOID TOMATOES FROM DIFFERENT ORIGINS[1]

E. W. LINDSTROM AND L. M. HUMPHREY
Iowa State College, Ames, Iowa

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INTRODUCTION

Because the tomato is so well adapted to regeneration in somatic tissue, especially in callus tissue, where chromosome doubling takes place occasionally, the artificial production of tetraploid forms is easily accomplished. By decapitating young tomato plants and allowing a wound callus to form on the cut stem, sprouts arise, some of which carry the doubled chromosome number as the result of a nuclear fusion in the callus (WINKLER 1916, JORGENSEN and CRANE 1927, JORGENSEN 1928, LINDSTROM and KOOS 1931).

The sources of our series of tetraploids are fundamentally four in number, but all arose from one or the other of the two tomato species, *Lycopersicum esculentum*, Mill. and *L. pimpinellifolium*, Dunal. While both of these species have twelve pairs of chromosomes, there is a marked difference between them, both cytologically and phenotypically, and the F_1 hybrid shows a small degree of sterility as evidenced by approximately 10–30 percent pollen abortion. The chromosomes of the *esculentum* species have a diameter at the second metaphase of 1.1 microns while the diameter of the *pimpinellifolium* chromosomes is only 0.8 microns. This fact, coupled with their great phenotypic difference, would seem to justify their separation into two species.

TETRAPLOIDS

Four kinds of tetraploids are to be considered as follows:

1. Tetraploid from haploid *esculentum* (through the diploid).
2. Tetraploid *esculentum*. Standard variety and variety F_1.
3. *Pimpinellifolium* tetraploids. Red Currant variety.
   a. From pure *pimpinellifolium* species.
   b. From unknown *pimpinellifolium* culture.
4. Tetraploids from F_1 hybrids of species cross.
   a. Red Currant × *ddpprryy* strain.
   b. Red Currant × variety Model (potato-leaf, dwarf).

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All of these tetraploids were produced by the decapitation-callus method, using petrolatum to cover the cut end of the stem. The percentage of tetraploid sprouts among the four sources was variable, ranging from none to 30 percent, with an average of about 10 percent. Chromosome doubling in the *esculentum* species was more frequent than in *L. pimpinellifolium*.

A brief description of each tetraploid type, coupled with its breeding behavior, will be given before the cytological studies are reported. Measurements of cell, nucleus and pollen sizes are given in a later section of the paper.

Tetraploid from haploid

From a haploid *esculentum* form (LINDSTROM 1929) which was practically wholly sterile, numerous markedly fertile diploid forms were produced by decapitation. From the latter were derived the tetraploids (figure 1). This type, possessing four identical chromosomes in each of the

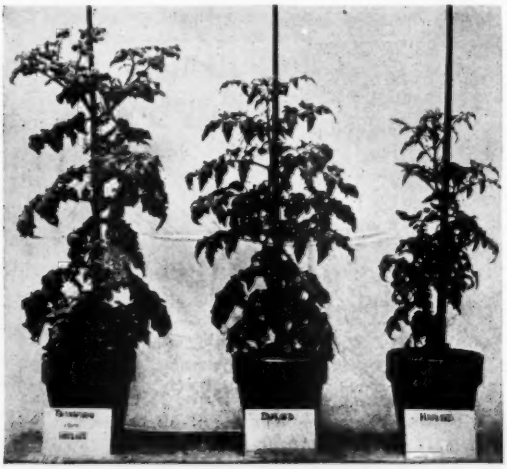

FIGURE 1.—Polyploid series from haploid. Tetraploid-diploid-haploid.

twelve sets and accordingly being absolutely homozygous, was typically sturdy and large in all its parts except the fruit. Although it showed about 80 percent normal pollen, it proved to be the least fertile of all the tetraploids. The set of seed was less than 10 percent that of its parental diploid. The interior of the fruits was characterized by hard, woody placental tissue. Three generations of progeny by self-fertilization have given only tetraploids of the parental form with the same low degree of fertility. Ordinarily only one to four fruits are produced by the best plants. This type is cross-sterile with diploid forms.

Tetraploid from L. esculentum

Included in the tetraploid series largely for cytological comparison, were two sets of these tetraploids. One arose from a standard pure strain and the other from a variety F_1 (Marglobe×dwarf, peach, ovate, compound-cluster). Since tetraploids of this sort have been reported by others, details are unnecessary. Our tetraploids showed medium fertility. Pollen abortion in the original forms was about 20 percent, and seed development about 40 percent normal. Later progenies of the hybrid exhibited more variation in these respects, but only tetraploids resulted from selfing the

FIGURE 2.—*Pimpinellifolium* diploid and tetraploid.

original tetraploids, whether of pure or hybrid origin. The genetic data from this variety F_1 tetraploid will be included in the section on "genetic observations."

Tetraploid from L. pimpinellifolium

From a homozygous strain of this fine-leaved, sprawling species (variety Red Currant), a series of tetraploids arose that are reported here for the first time (figure 2). These were characteristically (for tetraploids) larger and sturdier in stem, leaves, flowers and seeds. Pollen counts showed about 20 percent abortive grains. Fruit size was practically the same as in the diploid or perhaps a trifle greater, but not much. Why fruit size in these tetraploids is not appreciably greater is perhaps not according to expectation, but the fact that the seed set is only 30–40 percent normal is very likely the governing factor. These tetraploids also have only tetraploid progeny, with some variation in fertility although none of 90 plants has been completely sterile. This tetraploid form is completely sterile with the diploid species, when used either as the sire or the dam.

Another *piminellifolium* tetraploid has already been reported (LINDSTROM 1932) but since its cytological behavior is much the same as the others, no time will be given to it here, except to note that the fourth and

fifth generations from it continue to breed true, not only to the tetraploid condition, but also phenotypically. In the earlier report, it seemed as if there might be some *esculentum* "blood" in this form, but if so, no evidence of it has yet become apparent. It remains highly fertile for the most part,

FIGURE 3.—Species hybrid tetraploid and diploid.

only 3.0 percent of sterile plants being recorded in the fourth and fifth generations. Further tests have verified its complete sterility with both diploid species.

Tetraploids from F_1 of species cross

Because the chromosome (and pollen) sizes of the two species are significantly different it became particularly interesting to investigate the behavior of the hybrid between the two, both genetically and cytologically. Since the details of chromosome association in the diploid hybrid are un-

der investigation by one of the graduate students in the Department of Genetics, no report will be made of this herein.

Tetraploids were derived asexually by the callus method from two F_1 series of diploid plants (figure 3). The Red Currant variety was crossed by a pure strain of *esculentum* carrying the genes *ddpprryy*, as well as by the variety Model with the genes *ddcc* (dwarf, potato-leaf).

The tetraploid F_1 sprouts when grown, exhibited a somewhat intermediate type between the two species, with, however, the same characteristic dominance of the *pimpinellifolium* species in leaf, stalk, pubescence and fruit size that is found in the diploid F_1 plants. The tetraploid forms were, however, typically sturdier and larger in all respects than the diploid F_1,

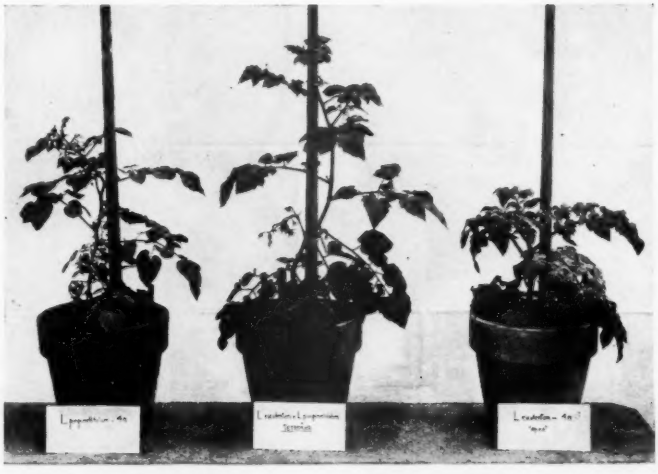

FIGURE 4.—Species hybrid tetraploid (in center) made by crossing two tetraploids.

even in the matter of fruit size although the difference here was slight. They exhibited the complete dominance of the known genes involved, all carried by the *pimpinellifolium* species. The same species F_1 tetraploid was also produced by crossing 4n *L. esculentum* by 4n *L. pimpinellifolium*, giving results identical with the preceding species tetraploid (figure 4).

The F_1 tetraploid plants were exceedingly fertile. Pollen abortion was approximately the same or slightly less than in other tetraploids, namely 10–20 percent. Seed set in comparison with the diploid was about 50 percent normal, the seeds being fully 30–40 percent larger.

The progeny from both of these tetraploid F_1 species crosses may be classed as highly fertile. Among 720 mature plants, only 3.7 percent were partially sterile and 0.4 percent failed to develop fruit with seeds. Prac-

tically all the others set fruit and seeds remarkably well, some being exceedingly fruitful. All progenies consisted only of tetraploid plants. Cytological tests were made of 20 partially sterile plants but all proved to have 48 chromosomes. Pollen counts showed a range of variability from 7 to 90 percent abortion, centering around 20 percent. Many of the plants with the high degrees of pollen sterility were nevertheless very fruitful.

The F_1 species tetraploid, like the other tetraploids, proved to be completely sterile reciprocally with either of its parental diploid species. Numerous crosses with diploids were made. The crossed fruits remained on

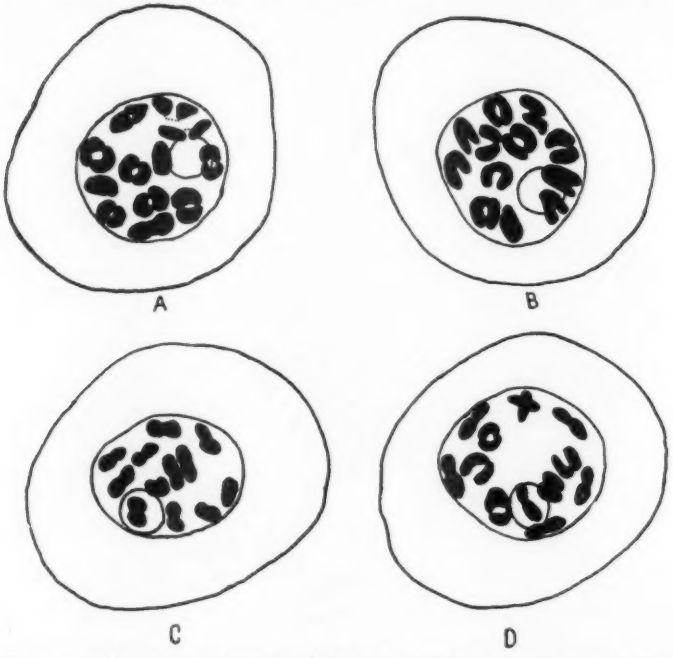

FIGURE 5.—Diakinesis. A—diploid from haploid. B—diploid of *L. esculentum*. C—diploid of *L. pimpinellifolium*. D—diploid of species hybrid. ×2500.

the plant, matured and developed a good color and size. But no fertile seeds were produced. The very small, thin, abortive seeds, often in great numbers, seemed to show that there had been a marked stimulation to growth of the ovules, but presumably the embryo failed to develop.

CYTOLOGICAL OBSERVATIONS

In general the cytological behavior of all the tomato tetraploids was surprisingly similar, but there were differences which are probably significant.

The material was killed in Allen-Bouin killing fluid and imbedded in paraffin. Sections were cut ten microns thick, and stained in iron alum-hematoxylin. All drawings were made at a magnification of 2500, with the aid of a camera lucida.

In the diploids, at diplotene, there is a partial separation or looping in the paired threads. As diakinesis is approached the chromosomes become much shortened and thickened, and usually the members remain attached at one or more points. In all the diploid cultures under consideration, except the species cross, the members of the bivalents nearly always remain

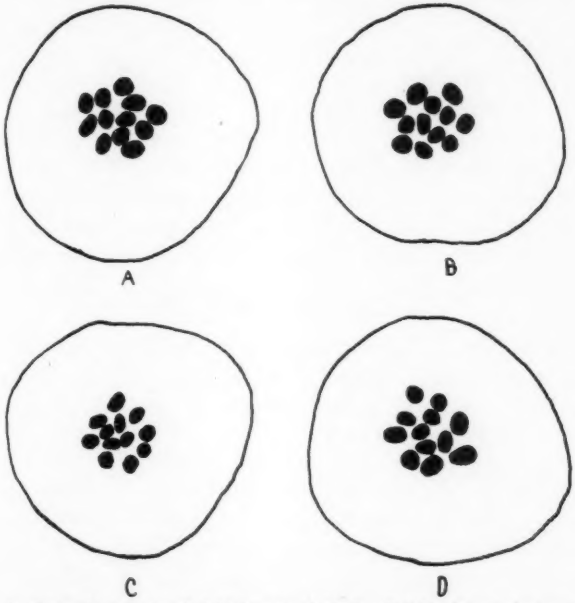

FIGURE 6.—Metaphase I. A—diploid from haploid. B—diploid of *L. esculentum*. C—diploid of *L. pimpinellifolium*. D—diploid of species hybrid. ×2500.

connected by at least one point and frequently by two (figure 5, A, B, C). These observations agree in general with those of JORGENSEN (1928), LESLEY (1926), and LINDSTROM and KOOS (1931) on diploid tomato material.

In the diploid of the species cross the association at diakinesis is not as close (figure 5, D). The univalent members of the bivalents are frequently separated at diakinesis, although the figure illustrated does not show this particularly. The pairs are often connected by one end or in loose bivalent rings. At metaphase, however, the bivalents, whether they were separated

or not, reunite into 12 nearly round bivalent chromosomes (figure 6, D), and the anaphase separation is normal.

In the species hybrid, during diakinesis, there is evident, in several of the bivalent pairs, a size difference between the members of the pairs. This might be expected since the average size of the *esculentum* chromosomes is over 30 percent larger than that of the *pimpinellifolium* chromosomes.

A certain general behavior in the tetraploids is apparent. In the prophase, at synapsis, there is a parasynaptic pairing of the four threads at least part of the time. Whether this is true in all cases is not known as yet. At early diplotene there is often visible a four parted thread, which would support the four strand synapsis, for it is very unlikely that the secondary split, presumably taking place at pachytene, would be visible at this stage, especially in material where the chromomeres are not distinct. Counts in early diplotene show approximately 12 twisted and looped threads, though there are sometimes more indicating that synapsis may sometimes be with double and not quadruple chromosomes. However, the number is usually 12 or close to it. From this stage on the tetraploids are considered separately, since individual variations are present in later meiotic stages.

Tetraploid from the haploid

This tetraploid is the most irregular in chromosome behavior through the stages from diplotene to the first metaphase. At diplotene there is the beginning of a separation of the quadrivalent sets of chromosomes into two parts, usually from the ends. In addition to this separation, there is also a looping within the bivalent elements, probably similar to the diplotene looping in the diploids. Careful observation of this tetraploid shows that at diplotene some of the chromosome threads are undergoing separation while others have not yet started. As early diakinesis is approached the remaining threads commence separation and those which began to split in diplotene have become separated into bivalents. The looping in the individual bivalent threads continues until a condition similar to that in the diploids is reached with these two differences: first, rings are practically never found, the association being end to end or side by side; and second, in some cases the separation is complete and the univalent members of the bivalents become entirely separated (figure 7, A). The diakinesis stages are somewhat irregular because the progress of the individual chromosomes in the cell is not equal. Some will have become separated into bivalents or even univalents, and have become much contracted while others are still in a tetravalent condition and not much shortened. This irregularity continues up to late diakinesis and only then will the separation into bivalents have been largely completed. Even then there

are sometimes groups of four that suggest a tetravalent condition. As metaphase is reached the 24 bivalent chromosomes line up on the equatorial plate and division occurs with regularity. JORGENSEN (1928) observed a condition very similar to this in his tetraploids, but LESLEY and LESLEY (1930) found strikingly different results in a tetraploid arising as a seedling instead of by decapitation. In their tetraploid there were largely

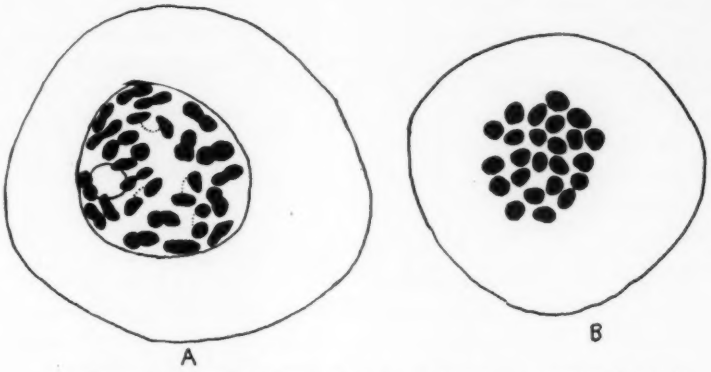

FIGURE 7.—Tetraploid from haploid. A—diakinesis. B—metaphase I. ×2500.

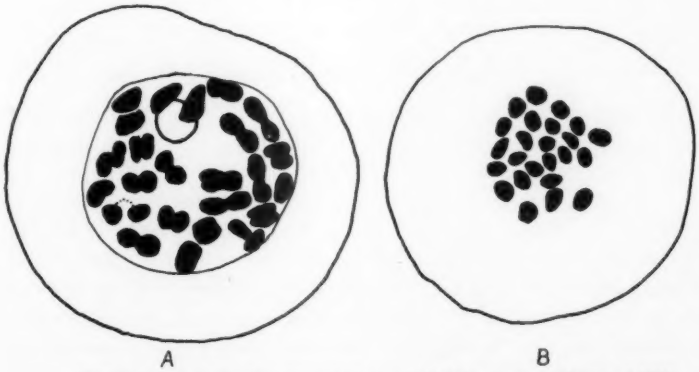

FIGURE 8.—*L. esculentum* tetraploid. A—diakinesis. B—metaphase I. ×2500.

tetravalents which persisted through metaphase I causing a very irregular anaphase and an irregular distribution of the chromosomes with consequent irregularities in the second metaphase. None of these irregularities was observed in our material, the behavior being very similar to that observed by JORGENSEN. At metaphase the chromosomes are nearly round and average 1.2 microns in diameter (figure 7, B).

Tetraploid esculentum

At diplotene there is evident the same sort of separation as was observed in the tetraploid from the haploid. With the *esculentum* tetraploid, however, all the threads separate at about the same time and subsequent behavior is more regular. In early diakinesis about half of the tetravalents have become separated into bivalents while the rest are still undergoing separation. In mid and late diakinesis there are 24 bivalents, their univalent members being usually connected at one end or at some other point. There are rarely ever bivalent rings, or complete separation of the univalent members. This condition was also observed in the diakinesis of a similar tetraploid form by JORGENSEN (1928) except that he saw occasional tetrasomes persisting to late diakinesis (figure 8, A). Metaphase I shows regularly 24 nearly round bivalents with a diameter of about 1.2 microns (figure 8, B).

Tetraploid pimpinellifolium

The diplotene of the tetraploid from the pure species is like the last one described in all respects. At early diakinesis, however, there are usually only bivalents, the separation from tetravalent to bivalent condition having proceeded more rapidly in this form. Throughout diakinesis the univalent members are nearly always connected by one end or side by side, but rarely ever become separated (figure 9, A). Metaphase I consists of 24 bivalents with an average diameter of about 0.9 microns (figure 9, B).

Tetraploid F_1 of species cross

The diplotene and early diakinesis stages in this tetraploid are similar to those of tetraploid *esculentum*. By mid diakinesis all the chromosomes are in bivalent condition. The members of the bivalent pairs may be connected at one or more places, and are also frequently separated completely (figure 10, A). However, at metaphase there are 24 bivalents somewhat more variable in size than in any of the other tetraploids (figure 10, B).

During late diakinesis there is frequently evident a difference in size between members of the bivalents identical with that seen in the diploid at the same stage. This suggests that smaller *pimpinellifolium* and larger *esculentum* chromosomes are pairing some of the time. Table 1 includes a brief description of each of the cultures described above.

Disjunction and anaphase I in tetraploids

The disjunction and anaphase I are normal in all the tetraploids except that occasionally a pair of chromosomes separates prematurely and proceeds to the poles ahead of the others. About as frequently one or more pairs lag behind the others and arrive at the poles late or not at all. There

is about 20 percent of such irregularity but it is doubtful if this has much to do with the pollen sterility common to the tetraploids, since only about 1 percent of the cells at second metaphase give counts of 23 or 25 chromosomes, and the second division is very regular. This is in agreement with JORGENSEN'S observations, but differs sharply from those of LESLEY and LESLEY (1930). They observed irregular second divisions and found varying numbers of chromosomes at second metaphase.

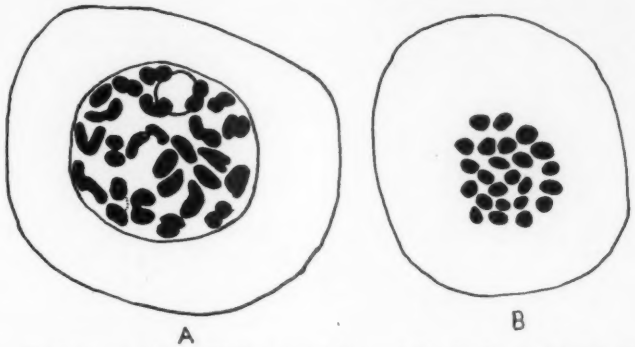

FIGURE 9.—*L. pimpinellifolium* tetraploid. A—diakinesis. B—metaphase I. ×2500.

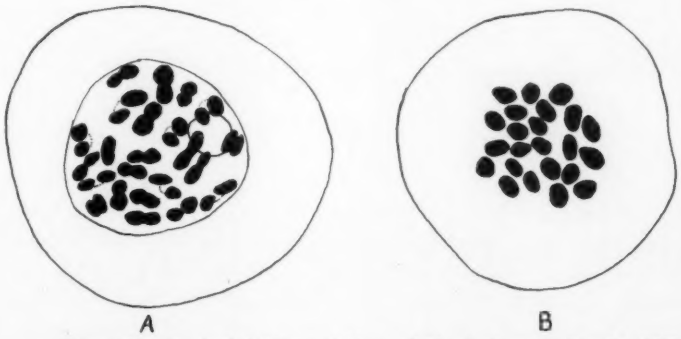

FIGURE 10.—Species hybrid tetraploid. A—diakinesis. B—metaphase I. ×2500.

MICROMORPHOLOGICAL OBSERVATIONS

Some interesting micromorphological observations were made which are summarized in table 2. At the second metaphase the chromosomes of *L. pimpinellifolium* have a diameter of 0.8 micron, and those of *L. esculentum* a diameter of 1.1 microns, a size difference of over 30 percent. The sizes of 1.2 microns given for the F_1 of the species cross in the table are for the first metaphase where the size is 0.1 to 0.2 micron larger than in the

TABLE 1

Cytological behavior of the various cultures.

CULTURE	DIPLOTENE	EARLY DIAKINESIS	MID DIAKINESIS	LATE DIAKINESIS	METAPHASE I	NATURE OF ASSOCIATION AT MID DIAKINESIS
Diploid from the haploid	Partial separation of the paired threads.	Chromosomes largely connected at ends, forming bivalent rings.	Twelve clear bivalents. All connected. A few flat rings, others connected at one end or throughout length.	Individuals of the bivalents more closely united. No loose formations.	Twelve bivalent units.	No pairs separated. A few connected by one end only. Two-thirds by both ends giving a flat ring. Remainder connected throughout. Association close.
Tetraploid from the haploid	Beginning of separation into bivalents, some looping in bivalent threads.	Partly bivalents and partly in diplotene condition yet. Behavior of individual tetrasomes not regular.	Largely bivalents. Usually some loosely associated tetravalents.	Usually all bivalents. Occasionally one or two groups of four loosely connected.	Twenty-four bivalent units.	Bivalents usually connected at one end or side by side. Frequently the univalent members are entirely separated—about one-sixth of the bivalents. Association fairly close.
Diploid esculentum	Looping of paired threads.	Chromosomes connected at the ends and at other points forming rings and other formations.	Twelve bivalents. Some flat rings—about one-fourth. Largely connected solidly at one end.	Twelve bivalents all closely united.	Twelve bivalent units.	No pairs separated. Association close.
Tetraploid esculentum	Beginning of separation into bivalents. Some looping in bivalent threads.	About half and half tetravalents and bivalents. Progression fairly regular.	Twenty-four bivalents. All closely united, only occasionally a pair stand separated. Union at one end or side by side.	Twenty-four bivalents.	Twenty-four bivalent units.	Pairs rarely separated. Association close.
Diploid pimpinellifolium	Looping of paired threads.	Connections at one or more points. Formations irregular.	Twelve bivalents. All univalents connected, nearly always by one end.	Twelve bivalents closely united.	Twelve bivalent units.	No pairs separated. Association close.
Tetraploid pimpinellifolium	Separation into bivalents uniformly proceeding. Looping in bivalent threads.	Usually already all bivalents.	All bivalents.	Twenty-four bivalents.	Twenty-four bivalent units.	Only occasional pairs completely separated into univalents. Paired usually end to end. Association close.
Species F_1 diploid	Looping in paired threads.	Loosely connected, usually by ends or one end. Large rings usually formed.	Rings, end to end, and frequently the univalents lie separated. Disparity of size often visible.	Association becoming closer.	Twelve bivalent units.	Occasional pairs separated. Others loosely connected. Association medium. Disparity of size frequently evident.
Species F_1 tetraploid	Beginning of separation into bivalents. Looping in bivalent	Partly tetravalents and partly bivalents. Progression uniform.	Bivalents. Only occasional tetravalents. Disparity in size of uni-	Twenty-four bivalents. Univalents usually all mated into bi-	Twenty-four bivalent units.	Bivalents sometimes separated into a pair of univalents. Disparity of size often visible. Association medium.

TABLE 2

Micromorphological characteristics of tomato species.

CULTURE	POLLEN DIAMETER	PERCENT DIAMETER	SECOND METAPHASE CHROMOSOME DIAMETER	PERCENT DIAMETER	CELL SIZE AT DIAKINESIS	PERCENT SIZE	NUCLEAR SIZE AT DIAKINESIS	PERCENT SIZE
L pimpinellifolium—2n	21.6	100	0.8	100	16.7	100	8.9	100
L. pimpinellifolium—4n	27.1	130	0.8	100	21.9	129	12.2	137
L. esculentum—Haploid	25.7	118	1.2	154	13.4	83	7.7	85
Diploid from Haploid	25.7	118	1.1	137	16.5	98	9.0	100
Tetraploid from Haploid	30.0	139	1.1	137	21.8	129	13.1	146
L. esculentum—2n	25.6	115	1.1	137	16.6	99	9.0	100
L. esculentum—4n	28.9	133	1.1	137	21.7	128	12.8	143
F_1 of Species Cross—2n	23.4	108	1.2 MI	154	16.5	98	9.0	100
F_1 of Species Cross—4n*	28.3	131	1.2 MI	154	21.5	127	12.1	137

* Made by decapitation of diploid of F_1 of Species Cross. All measurements in microns.

second metaphase. Figure 6 shows comparative size differences at the first metaphase.

Pollen diameter was found to vary both with the species and with the number of chromosomes present. *L. pimpinellifolium* had the smallest pollen with a diameter of 21.6 microns, while the tetraploid from the haploid had the largest with a diameter of 30.0 microns. When the chromosome number is increased from 2n to 4n there is a corresponding increase in the size of the pollen grains. In *L. pimpinellifolium* the increase was about 30 percent and in the other forms approximately 20 percent. The F_1 of the species cross has a pollen diameter almost exactly intermediate between the respective parents in both diploid and tetraploid.

The cell and nucleus size of the pollen mother cells at diakinesis was found to vary directly with chromosome number. The sizes were the same in both species. The haploid had the smallest cell and nucleus diameter, 13.4 and 7.7 microns respectively. The diploids with slight variations had cell and nucleus sizes of 16.5 and 9.0 microns respectively, and the tetraploids had cell and nucleus diameters of approximately 21.7 and 12.5 microns respectively.

The micromorphological and gross morphological characters of the cultures have the same trend except in the matter of cell size. Morphologically *L. esculentum* is larger and coarser than *L. pimpinellifolium* in most respects (fruit, seed, trichome, leaf, stem, flowers, and stature). The same relation holds for their pollen and chromosome sizes. In general the tetraploids are larger than the diploids in their morphological characteristics. This is also true of the relative sizes of the pollen grain, as well as of the cell and nucleus at diakinesis.

GENETIC OBSERVATIONS

The more critical observations in this report center around the genetic behavior of the species cross in which the chromosome sizes are different. Four chromosomes were marked genetically (*Dd*, *Rr*, *Yy* and *Cc* genes). The *pimpinellifolium* species carried all the dominant genes. Only F_2 data are reported, since the tetraploid multiple-recessive form, necessary for a backcross, was not then available. Linkage data are not discussed, since the numerous modifying genes of the *pimpinellifolium* species prevented accurate classification of the *Pp* genes which are linked with the *Dd* genes.

Knowing that the chromosomes of the two species differ cytologically in size, and genetically in many genes, and that they assort from a bivalent condition at diakinesis, one might fully expect a pairing of like chromosomes at this stage. This would give bivalents of the composition *AA* and *aa* for any given set of genes and would result only in *Aa* gametes. Such is not the case, for segregation occurred in every one of the four genes tested (table 3). Evidently unlike chromosomes (from different species) or parts of chromosomes, pair and emerge as bivalents at diakinesis.

TABLE 3
Tetraploid F_2 segregation.

	D	*d*	*S*	*s*	*R*	*r*	*Y*	*y*	*C*	*c*	FERTILE	PARTIALLY STERILE	STERILE
Species hybrid													
RC×*dpry*	328	5			376	19	382	13			375	21	0
RC×*dc*	555	23							545	33	335	8	2
Total	883	28			376	19	382	13	545	33	710	29	2
Ratio	32:	1			20:	1	29:	1	17:	1			
Esculentum hybrid	258	4	235	7	224	8					208	26	12
Ratio	64:	1	34:	1	28:	1							

Apparently this unexpected result traces to the behavior of these chromosomes in the prophase stages when they are associated as tetrasomes. Were there merely random assortment of the four chromosomes (or genes *AAaa*) here, a 1 *AA*+4 *Aa*+1 *aa* proportion of gametes would result, giving a 35:1 phenotypic F_2 ratio. The data, however, exhibit a higher percentage of recessives than this in every case.

In preliminary reports of pure *esculentum* tetraploids, the 35:1 ratio seems to be the rule (SANSOME 1931). Our own data on such tetraploids might be considered as showing an approach to the same condition (table 3), although the numbers are relatively small. The *Dd* results cannot be

depended upon with certainty because of the difficulty of classifying when four, three, two, one or no dwarf genes are operating.

The species hybrid tetraploids must be considered as exhibiting a significant departure from this 35:1 ratio, particularly in the *R*, *Y* and *C* genes, where classification is certain. Such departures could be explained on a theoretical random assortment of eight chromatids of any tetrasome, approaching as a limit the 21:1 ratio of dominants to recessives. If the genes were far removed from the spindle fibre attachment, this extreme ratio might well be approached with the help of crossing over. There is no desire at present to stress this point since more critical back-cross data will be available in the future.

The important point is that both genetic and cytological evidence demonstrates that chromosomes which are quite unlike will still pair (and very likely cross over). In a tetraploid such as the one dealt with, we might expect a preferential pairing of the two like chromosomes from each species. Evidently something stronger than the attraction of ordinary Mendelian genes influences chromosome pairing. Apparently the two tomato species, one the domesticated form and the other approximating the wild species, are still closely related through their chromosomes even though they are so different phenotypically. Is it not possible that more fundamental genes (in which the two species are still alike) exercise more attraction for their chromosomes than the genes now differentiating the two forms? Or is there some other agency that determines chromosome pairing?

From his studies of tetraploids in Lycopersicum, *Solanum nigrum*, *S. luteum* and *S. nigrum*×*S. luteum*, JORGENSEN (1928) was also forced to a similar conclusion. He maintained that "The capacity for conjugation cannot, as is usually assumed, be considered a reliable measure of the degree of identity of the chromosomes entering meiosis. Other factors than the chromosomes themselves may influence the process of conjugation."

In our tomato tetraploids, particularly the tetraploid from the haploid, where all the four chromosomes in any one set are identical, strict tetrasomic association would be expected, as is the case in Datura. While there was evidence of such tetrasomic conjugation in the prophases, the striking thing was the very evident disomic condition at diakinesis. Something outside the chromosomes would seem to be causing the bivalent association at this stage.

Both genetic and cytological evidence then, point to tetrasomic association in prophase and to disomic association at diakinesis. The genetic evidence proves that unlike chromosomes (such as those differentiating the two tomato species) or unlike parts of chromosomes (after any crossing over in the tetrasomes) pair at diakinesis. This is not to be expected on

the usual concepts of chromosome pairing, and suggests that simple gene attraction is not the cause of chromosome pairing at diakinesis in tetraploids.

SUMMARY

Tetraploids from four different sources involving the two species of the tomato, *L. esculentum* and *L. pimpinellifolium*, were produced by the asexual, decapitation-callus method. Descriptions, breeding behavior and fertility of these forms are reported.

The least fertile tetraploid was the one derived originally from a haploid form. This type in which all four chromosomes in each of the twelve sets are identical, produced fruit sparsely, and seed development was less than 10 percent normal, although the plant is very sturdy and pollen development excellent.

The most fertile tetraploid was the hybrid between the two species. The pure *esculentum* and pure *pimpinellifolium* tetraploids were intermediate in fertility.

All the tomato tetraploids reported were strikingly similar in two important respects. First, all bred true to the tetraploid condition. Diploids or triploids were not produced. Second, all showed complete, reciprocal sterility with the diploid forms of either species. Being phenotypically different from their parental types, being self-fertile and cross-sterile with all diploids, they offer the possibility of an origin for new tomato species.

Cytologically, all the tetraploids were surprisingly similar in their basic chromosome behavior, a fact which was not expected. At metaphase they usually exhibit 24 bivalents, the tetravalent condition in early prophase having largely disappeared by late diakinesis and metaphase I. There is a difference in the rate of change from tetravalents to bivalents in the various tetraploids.

Disjunction of the bivalents at anaphase was normal, except that occasionally a pair of chromosomes separates prematurely and proceeds to the poles ahead of the others or lags. This apparently does not disturb the end results for less than 1 percent of the cells at second metaphase had more or less than 24 chromosomes. The second division was very regular.

Chromosome size at second metaphase was different in the two species, the *esculentum* chromosomes being 1.1 microns and the *pimpinellifolium* chromosomes 0.8 micron in diameter. There was cytological and genetic evidence that unlike-sized chromosomes in the species cross pair in diploids and tetraploids. Chromosome association in the diploid of the species cross was slightly looser than in ordinary diploids.

The size of the pollen grains varied with the species as well as with total chromosome number. Pollen-mother-cell and nuclear sizes, however, were approximately the same for all diploids; and the tetraploids were all alike but were about 40 percent larger.

Genetically four different chromosomes were tested in the species hybrid tetraploid. All the four genes concerned showed segregation, the F_2 ratios, however, being less than the 35:1 ratio expected on the random assortment of the four chromosomes in any one set. The higher percentage of recessives fits the hypothesis of a more or less random assortment of eight chromatids in any tetrasome.

The genetic and cytological results in this species hybrid tetraploid give some evidence that chromosome pairing is not in general governed by mere gene attraction since the gene differences between the two species were numerous and yet unlike chromosomes apparently paired at diakinesis.

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SEX-LINKED GENES IN THE FOWL

JOHN W. MACARTHUR
University of Toronto, Toronto, Ontario, Canada

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INTRODUCTION

A study of the linkage relations obtaining among certain sex-linked genes of the fowl was begun by the author in 1925, the investigation having been first stimulated by PUNNETT's summary (1923) and the earlier work of GOODALE (1917), HALDANE (1921) and SEREBROVSKY (1922). After a preparatory detailed survey and several test matings of breeds to discover those containing the most favorable contrast of characters, chief attention was directed to reciprocal crosses between Columbian Plymouth Rocks and Gold Campines.

The former breed carries the dominant sex-linked genes for silver, slow feathering, light shank color, and light eye color, and lacks the barring factor, reputedly confusing in classification. The Gold Campine, on the other hand, carries all the corresponding recessive allelomorphs: gold, fast feathering, dark blue shanks, and dark brown eyes, as well as a number of additional contrasting characters of interest in other connections, such as those involved in down colors and patterns, ear-lobe and egg colors, hen-feathering in the male, etc.

The sex-linkages found were published in a preliminary note (MACARTHUR 1927); the values there reported have been confirmed in substance in the following years.[1] Meanwhile the number of known sex-linked genes has been increased and our knowledge of their localization in the sex chromosome has been extended by a number of other investigators (SEREBROVSKY and WASSINA 1927, WARREN 1928, HERTWIG and RITTERSHAUS 1929, RITTERSHAUS 1930, HERTWIG 1930). As might be expected from the large size of this chromosome (HANCE 1926), the number of sex-linked genes in the fowl is relatively large, seven at least having been determined up to the present, a greater number than is known in either of the autosomal linkage groups.

CHARACTERS AND FACTORS STUDIED

This report deals chiefly with the linkage relations between four pairs of sex-linked genes, of which the last, controlling light or dark iris color, is so studied for the first time:

[1] The carrying out of this project has been made practicable by the funds and stocks provided through the Department of Biology and the Poultry Department, and by facilities and direct help furnished by C. G. MACARTHUR at Bowmansville.

1. Silver versus gold (S, s)

These well known factors are easily distinguished in the ground color of the down of even the darkest chicks from the crosses listed below as 3, 4, 5, and 6. More difficulty and delay in classification in the first two crosses caused them to be early discontinued. Though silver is clearly dominant over gold, practically every *Ss* male bore numerous feathers partly gold, due probably to somatic segregation as described by SEREBROVSKY (1926).

2. Slow versus fast feathering (K, k = Sl, sl)

The rate of feathering is distinguishable with comparative certainty even in F_2 populations by the development attained by the wing quills at hatching and the length of the wing and tail feathers on the tenth day after hatching (WARREN 1925, 1928, 1930).

3. Light versus dark dermal melanic pigmentation in the shank (D, d = Y, y)

The deep mesodermal pigment beneath the scales is slow developing and in young chicks is often quite masked at first by yellow lipochrome in the skin and for some time or permanently by the black epidermal pigment which occurs especially in the darkest downed chicks (crosses 1 and 2). At or about three months of age, as later rechecks show, the shank color becomes fixed and may be safely, and with few exceptions reliably, classified as either dark (slaty blue or green) or light (white, light blue, or yellow or light green)—(DUNN 1925). Since dark plumage colors blacken the shanks to a troublesome degree, crosses such as 5b and 6b producing pale or restricted black downs, are especially favorable.

4. Light (yellow, orange to bay) versus dark (brownish black) iris color, (Br, br)

That the main genes controlling this contrast of eye colors are a sex-linked pair is concluded from their typical criss-cross inheritance, as in the reciprocal matings 5 and 6, and from their linkage relations. Such a conclusion has only been gradually won, after a period of confusion and change of views calling to mind the history of dark shank color inheritance.

The range and breed distribution of eye colors in fowls were described by DAVENPORT (1906), who observed that black eye tends to become associated with black plumage. Iris colors were classified histologically by BOND (1919) after the early plan devised for mammals, as: (1) simplex (*pearl*), with no anterior pigment and only a rather opaque stroma to obscure the posterior uveal color in hexagonal cells; (2) duplex, with one layer of anterior pigment, either (a) *yellow* or *red*, the round granules concentrating around connective tissue and in muscle fibres, or (b) *brown* or

black, the granules packed in a rich plexus of branching cells; and (3) the doubly pigmented triplex, as in Silky, where a *black* plexus overlies a deeper and separate yellow layer and the melanism is wide spread in corium and other mesodermal tissues. This order was considered in general as the order of ascending dominance or epistasis. In fact, however, it seems that but a few of the published crosses exhibit such autosomal dominance of the brown over the red or yellow eye.

DAVENPORT (1906) noted in F_1 hybrids from Black Minorca ♀ × Dark Brahma ♂ the imperfect dominance of yellow iris over brown. From matings of pearl-eyed Malay or yellow-eyed Buff Orpington hens with Black Orpington cocks BOND (1919) obtained chicks with black down and irides dark at first; but the cockerels became light-eyed at maturity by the gradual withdrawal or atrophy of the surface layer of branching cells containing the brown granules, while the pullets remained dark-eyed. He suggested that this change was sex-limited, but apparently did not make the corresponding reciprocal crosses to exclude the possibility of ordinary sex-linkage. PUNNETT (1923, pp. 157 and 58) found dark eye dominant in Sebright × Hamburgh, but both dark eye and dark shank recessive and apparently sex-linked in the F_1 generations from reciprocal crosses of Brown Leghorn × Black Langshan; also sex-linked was melanism in the Silky × Brown Leghorn matings. Since the main object of this work is to collect, compare, and interpret observations, it is necessary to dissent from PUNNETT's reference to Spangled Hamburghs and Campines as "dark-eyed and purple-faced" breeds; at least our stock of the former was orange-eyed, and neither breed had the purple in the face, comb and earlobe that is found in Silky and some Sebrights. In our crosses the sex-linked type of inheritance has been generally followed.

The chick iris color is evidently somehow influenced by epidermal plumage melanins, and the definitive color, either dark or light, becomes established only with advancing maturity. The presence or absence of the deep brown pigment in the adult iris is not then constantly associated with any particular color in the plumage, shanks, or skin, for in our segregating populations, as in pure breeds of fowls, either the dark or the light eye may be found with either of the skin and all the shank colors and with a great diversity of plumage colors and patterns. Dark-eyed F_2 birds, for instance, range in plumage color, either in the gold or the silver series, from black, through Campine marked, to the flecked, and to the nearly pure white of a very restricted Columbian pattern, and they may have shanks of any of the four main color groups. The breed correlation between black feathers and dark shanks and eyes is also broken in many exceptions, where black fowls have only one, or neither of the latter characters. Conversely, light plumaged F_2 birds with restricted black some-

times have one, the other, both, or neither of them. These crosses do not show what effects, if any, are exerted on iris colors by genes for the various recessive whites, barring, and a number of other colors and patterns. Since the pale birds from crosses 5b and 6b (p. 214) do not develop deep brown eyes, the fundamental color and certain melanin forming and extending factors are possibly prerequisites for the fully dark iris. In this material there was evidently no inhibitor of melanin common to shank and iris mesoderm, but the situations in Silkies and canaries and the known interrelation in mammals between eye and hair colors, hint that common distributing and interacting factors for these pigments in their different sites may be expected here.

5. Li li

Some observations of the relations of a fifth pair, *Li li*, controlling light or brown in the down have been gleaned from my notes since the literature on the sex-linked down color and pattern factors came to my notice.

CROSSES MADE

1. Brown Leghorn ♀, *D Br k s*, ×*Silver Campine* ♂, *dd brbr kk SS*

The F_1 chicks were all silver and fast feathering, with black pigments so extended and intense as to conceal both parent down patterns, and make the shanks nearly black; the females were brown eyed, the males orange eyed. In the small F_2 population the females segregated for silver and gold; eye and shank color segregations could be followed in only a few favorable cases grown to maturity.

2. Silver Campine ♀, *d br k S*, ×*R. I. Red* ♂, *DD BrBr KK ss*

The F_1 chicks were either uniform red-brown or campine patterned, the males silver and females gold, and both sexes slow feathering, with bluish-white shanks and slightly darkened yellow eye. In F_2 both sexes segregated for silver and gold, only the females for the remaining factors; silver here appears more frequently linked with dark shanks and dark eyes.

3. Gold Campine ♀, *d br k s*, ×F_1 ♂, $\frac{D\ Br\ K\ s}{d\ Br\ k\ S}$, *ex R.I. Red* ♀ ×*Silver Spangled Hamburgh* ♂

The mating producing the male was first made to study the reported sex-linked spangling character (LEFEURE and RUCKER 1923), but this pattern behaved practically like an autosomal dominant.

4. Columbian Plymouth Rock ♀, *D Br K S*, ×*Silver Campine* ♂, *dd brbr kk SS*

The F_1 chicks were all light to dark gray in down with light silver faces; the females being fast feathering, dark shanked and dark eyed, the males

slow feathering, light shanked and light eyed. The F_2 generation segregated for these three pairs.

5. Columbian Plymouth Rock ♀, D Br K S, × Gold Campine ♂, dd brbr kk ss

Both breeds carry black, which is non-extended in the Columbian pattern (*ReRe*), but the Gold Campine (unlike the Silver Campine) strains used differed among themselves in some melanin extension factors (whether $E^m e^m$, or tine-atine—SEREBROVSKY 1926—or others), for in 1926, both in this cross and its reciprocal, 6 below, some pairings produced F_1 chicks of both sexes in two equal and very distinct classes:

a. Dark, slaty (extended), dove-gray to blackish-gray, chicks with light faces (gold in ♀ ♀, silver in ♂ ♂), but with no visible down striping.

b. Pale, non-slaty (restricted), all showing a distinct, but more or less modified Campine mottled or 4-striped pattern in the down. The females have less intensely brown eyes than in 5a.

c. New Gold Campine stocks obtained in 1928 from other sources, however, produced when crossed all dark chicks, 51 ♂ ♂ and 52 ♀ ♀, with sex distinctions as in 5a.

In crosses 5a, 5b, and 5c the F_1 females are all *d br k s*, the males $\frac{D\ Br\ K\ S}{d\ br\ k\ s}$, and the entire F_2 populations segregate for all four of the sex-linked character pairs. Reciprocal back-crosses and numerous F_2 *inter se* matings were made from 5c.

6. Gold Campine ♀, d br k s, × Col. P. R., ♂

From this cross appeared both

a. Dark, slaty gray chicks with silver faces, and

b. Pale chicks with a Campine-like pattern of brown, black, and light stripes and flecks on the head, cheeks and back. All chicks, whether dark or pale, male or female, were slow feathering and finally developed orange eyes (with occasional faint brown dotting or rays) and light blue or white shanks. In the F_2 only females showed segregation and new crossover classes for the sex-linked factors.

CUTLER (1918) noted, but probably misinterpreted as somehow sex-linked, similar dark and pale classes in hybrids (all males) from Gold Campine ♀ × pheasant ♂.

DATA OBTAINED

A. Segregation ratios for the different gene pairs

From these crosses the ratios of the dominant and recessive allelomorphs were separately noted. In some matings as 1, 2, and 6, only females segre-

gated for some or all of the pairs. This fact, and the accumulating losses due to pests and natural deaths, account for the differences in totals. The sex, wherever doubtful, was ascertained by dissection, the sex ratio in F_2 being 269 ♂♂; 289 ♀♀. The other ratios totalled from all the crosses approximate the 1:1 expected, there having been classed: 334 silver: 359 gold; 309 slow feathering: 301 fast feathering; 186 light shanked: 201 dark; and as regards eye color, 191 reddish: 156 brown. No tendency was observed to a sex limitation in iris color or other genes.

B. Linkage relations between the four pairs of sex-linked genes

Crossover results were taken from either the coupling or repulsion phase or both for all six groupings of the four pairs when they are taken two at a time. The bulk of the data is from the coupling phase in crosses 5 and 6. Tabulated separately in the notes for each phase in each of the crosses the ratios are here merely totalled (table 1). The percentages of crossing over were calculated without correction for differing viabilities of the classes.

TABLE 1

Showing the number and percentage of crossovers occurring between the series of sex-linked genes.

FACTOR PAIRS	SEGREGATION		TOTALS	CROSSOVER PERCENTAGES AND THEIR STANDARD ERRORS
	ORIGINAL COMBINATIONS	CROSSOVER COMBINATIONS		
Ss and *Kk*	520	69	589	11.7±1.3
Ss and *Dd*	200	177	377	46.9±2.6
Ss and *Br br*	210	164	374	43.8±2.6
Kk and *Dd*	206	185	391	47.3±2.5
Kk and *Br br*	171	157	328	41.8±2.7
Dd and *Br br*	215	83	291	27.5±2.6

The data indicate linkages of different intensities and significance. Shank colors show so high a percentage of crossing over with silver and gold (46.9 ± 2.6), and also with rate of feathering (47.3 ± 2.5), that assortment is practically free in both cases. Silver-gold and rate of feathering, however, are clearly and closely linked, with only 11.7 ± 1.3 percent of crossing over; this value is much smaller than the 19 percent found by SEREBROVSKY (1922), or the 14 percent of WARREN (1928), and more closely approaches the 10 percent suggested as most likely by HERTWIG and RITTERSHAUS (1929).

Iris colors appear to show a definite though rather loose linkage with both silver-gold and rate of feathering, the crossover values being 43.8 ± 2.6 and 41.8 ± 2.7 percent respectively.

Between shank colors and iris colors another relatively close linkage is evident, for the crossovers are significantly low (27.5 ± 2.6 percent).

C. Linkage relations with other sex-linked genes

For reasons stated, barring was intentionally, though perhaps needlessly, avoided in this study; neither spangling nor ear-lobe color (WARREN 1928b) exhibited definite sex-linked behavior; and, though a relatively large number of F_2 chicks were found from crosses 5 and 6 which showed a head spot, and a 4-lined striping, phenotypically resembling those controlled by the *ko* and *st* genes, we have not been able to show in our material that either was sex-linked or associated with sex-linked genes.

The *Li li* factors which inhibit or produce brown in the down of non-black chicks (HERTWIG 1930, HERTWIG and RITTERSHAUS 1929) were not known to us when our notes were taken, but from the first it was clear that the F_2 campine-patterned and other markedly brown chicks were usually, but not invariably, gold and fast feathering. Of 52 such chicks from crosses 5a and 5c, 36 were gold and 16 silver, 35 fast feathering and 12 slow feathering, where equality would be expected, if sex linkage with *li* were not involved. In cross 6a, the 13 F_2 chicks which had been classified as campine-like were all (with one possible exception) females, as would be expected in dealing with the sex-linked *Li li* factors. Moreover, the corresponding class of light unstriped chicks, which are among those lacking brown in the down, show an equally marked excess of silver, slow feathering, light shanked and light eyed birds. A rough estimation of *li* linkages from these classes would seem justified since they result from autosomal segregation; the crossing over is about 31 percent between *S* and *Li*, 32 from *K* to *Li*, 16 from *D* to *Li*, and 23 from *Br* to *Li*. Naturally no great stress is to be laid on these figures in the quantitative sense.

D. Four-point tests

In crosses 5 and 6 the characters are suitably disposed in the parents and the F_2 populations reared large enough for a fairly reliable determination of the number of the single, double, and triple crossovers occurring in the F_1 male (table 3).

Among the 272 F_2 fowls, 96 (35.3 percent) represent original combinations, 120 (44.1 percent) single crossovers, 50 (18.4 percent) double crossovers, and 6 (2.2 percent) triple crossing over. The great frequency of double and triple crossovers is attributable to the great cytological length of the sex chromosome and the wide dispersal upon it of the loci studied. The triple crossovers are unexpectedly numerous; since all five *d Br k S* phenotype individuals were entered from a single small population, one of the first to be classified, it seems likely that the value given is too large.

In these tests the crossover percentages approximate those obtained from the whole body of data: *D-Br*, 29.9; *Br-K*, 47.7; *K-S*, 9.9; *Br-S*, 49.6; *D-K*, 44.4; and *D-S*, 46.3.

TABLE 2

Showing the frequencies of the different phenotypes segregating in the F_2 generation from $F_1\, d\ br\ k\ s \,♀ \times F_1 \frac{D\ Br\ K\ S}{d\ br\ k\ s}\,♂$, *and among the female progeny from* $F_1\ D\ Br\ K\ S ♀ \times F_1\ ♂$.

NON-CROSSOVERS	NUMBER	PERCENT	
D Br K S	48		35.3
d br k s	48		
Single crossovers			
d | *Br K S*	22	11.1	44.1
D | *br k s*	8		
d br | *K S*	40	29.3	
D Br | *k s*	40		
d br k | *S*	5	3.7	
D Br K | *s*	5		
Double crossovers			
d | *Br* | *k s*	23	14.4	18.4
D | *br* | *K S*	16		
d br | *K* | *s*	3	1.8	
D Br | *k* | *S*	2		
d | *Br K* | *s*	4	2.2	
D | *br k* | *S*	2		
Triple crossovers			
d | *Br* | *k* | *S*	5		2.2
D | *br* | *K* | *s*	1		
Total	272		100.0

The numbers in the different classes unfortunately do not settle definitely the sequence of the genes, but silver appears to be more distant than rate of feathering from both *D* and *Br* loci, and *Br* would lie to the left of *D*.

SEX CHROMOSOME TOPOGRAPHY

Judging from the entire data here supplied, the loci of *Ss* and *Kk* are somewhat less than 12 units apart. From the average of all the determinations the *Ss* locus is separated by at least 44 units and that of *Kk* by at least 42 units from *Brbr*, and both *Ss* and *Kk* by still greater distances than these from *Dd*. Again, *Dd* and *Brbr* are about 27.5 units distant. The observed *D-K* and *Br-S* crossover values agree well with calculated expectations. These and other recent data (HERTWIG 1930) seem to justify reversing the positions of *K* and *S*, suggested by SEREBROVSKY and WASSINA (1927) and WARREN (1928). Such meagre evidence as the material furnishes suggests that the locus of *Li li* may be nearer to the center of the chromosome, rather than at the extreme right end (HERTWIG 1930). The

map localization of the four factor pairs might be represented as follows:

D	27.5	*Br*	42	*K*	12	*S*
d		*br*		*k*		*s*

The factors described above are apparently favorably distributed along the chromosome, the *Br br* genes helping to fill in the long empty central portion, and bridge over the wide gap between *D* near one end and *K* and *S* toward the other extreme. But it is still impossible to claim that this or any other present mapping of the sex chromosome is more than tentative, as compared with maps in Drosophila, maize, tomato, sweet pea, etc., though this is perhaps the best localized large group of linked genes that is offered in any vertebrate animal.

If we add barring, *B*, and the new genes for head spot, *ko*, and brown-indown *li* (HERTWIG 1930, RITTERSHAUS 1930), we find uncertainties and inconsistencies also in the localization of these. Combining the data of all authors, and retaining the location of *ko* and *li* proposed by their discoverers, the following linear order and approximate spacings are suggested, merely as a working hypothesis:

B	*D*	23	*Ko*	*Br*	42	*K*	12	*S*	17	*Li*
b	*d*		*ko*	*br*		*k*		*s*		*li*

This provisional arrangement is based upon *one* interpretation of the crossover percentages contributed by the various workers in this field (HALDANE 1921, AGAR 1924, SEREBROVSKY and WASSINA 1927, WARREN 1928, HERTWIG and RITTERSHAUS 1929, HERTWIG 1930, RITTERSHAUS 1930). This data has been reviewed recently in the four last named papers, and further summary is unnecessary.

A critical examination and comparison of the findings shows that where several determinations of a value have been made, their agreement in details is generally good, and often excellent. Yet there are doubtful general points and not a few evidences of internal disharmony in the proposed arrangement as a whole. Such loose linkages have had to be used in the map construction that they provide no very accurate estimate of distances between genes or even of the actual linear order of the genes. Even the point test is no panacea in such a difficulty.

The precise linear relations of *B* and *D* are not sure, since, owing to the masking influence exerted by barring over shank color (WARREN 1928a), their positions and distance are necessarily found only indirectly through third genes. Further and new tests of the linkage of *B* and *D* with the not too distant head spot and iris color genes will doubtless greatly improve the known topography of the left half of the chromosome.

Crossover values between *ko* and *br* have never been taken, but their linkage is theoretically close. When suitable stocks have been found for

making this determination it will be interesting to see how well observation checks with theory in the central portion of the map.

The greatest quantitative discrepancies concern the location of the newer, less investigated genes, particularly *li*. The reported *B-Li* and *D-Li* crossover values are so low—40 and 31 percent respectively—as to appear decidedly anomalous and unlikely, if these genes lie at opposite ends of a chromosome over a hundred units long. *Li* may need to be relocated; in fact the map would fit the present data nearly as well if the *K S Li* section were inverted.

To ascertain the proper order of *K* and *S* in the right half of the chromosome, their linkages will have to be studied with genes that are closer than are *B* and *D*, and perhaps even closer than *Ko* and *Br*.

SUMMARY AND CONCLUSIONS

From Columbian Plymouth Rock×Gold Campine, and other interbreed crosses, the linkages were studied of the four pairs of sex-linked genes controlling silver or gold in the down and plumage (*S*, *s*), slow or rapid feathering (*K*, *k*), light or dark dermal shank color (*D*, *d*), and orange or brown iris color (*Br*, *br*), the last named pair being new in such studies.

The percentages of crossovers, with their standard errors, found were: *Ss-Kk*, 11.7 ± 1.3, *Ss-Dd*, 46.9 ± 2.6, *Ss-Br br*, 43.8 ± 2.6, *Kk-Dd*, 47.3 ± 2.5, *Kk-Br br*, 41.8 ± 2.7, and *Dd-Br br*, 27.5 ± 2.6.

These values, though not in full agreement with results of a large four-point test, indicate the most likely linear order and the approximate spacings of these genes to be:

Dd 27.5 *Br br* 42 *Kk* 12 *Ss*

A very provisional mapping of the sex chromosome, on the basis of all the published data and including the loci of barring, *B*, head streak, *ko*, and brown-in-down, *li*, genes, demonstrates a great number of uncertainties and inconsistencies in the proposed arrangement requiring correction by further investigation.

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THE NATURE OF SIZE FACTORS IN DOMESTIC BREEDS OF CATTLE

PAUL WALLACE GREGORY
University of California, Davis, California

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During the past thirty years almost all the investigators who have been interested in size or size inheritance in animals have given some consideration to the nature of the genetic agencies or factors which control size. Even at the present time the question has not been absolutely settled as to whether genes which affect size are general or specific in their nature for the mammalia as a class. Investigators agree that general factors do exist which affect the growth of all parts of the body in the same direction and to proportional degrees. The mooted question is whether or not specific growth factors which affect local areas and units or specific tissues within the individual actually exist. Professor CASTLE (1914 and 1922) from numerous correlation studies gave evidence that factors which control growth in the rabbit are general in their action and affect all parts of the body in the same general direction and to a proportional degree. Moreover, CASTLE found that the lengths of the different bones within an individual are highly correlated, and that all the bone lengths he studied were correlated to a high degree with body weight. In addition to this, CASTLE found for the rabbit that the length of the ears is highly correlated with body weight. These studies seem to show for the rabbit that muscular development is definitely correlated with skeletal development in some manner, and that both are controlled apparently by the same system of general growth factors.

In the studies made by CASTLE, muscle was not considered as a distinct tissue but was included in the total body weight. In 1918 WRIGHT analyzed the data presented by CASTLE in 1914 and came to the conclusion that for the most part, the factors which control bone length are general. However, he concluded that in the development of certain bones and groups of bones there was a slight amount of variation which was controlled by specific growth factors and was consequently independent of general growth factors. In CASTLE's later analyses (1922) this specific action of growth factors was not apparent from his method of analysis. By means of path coefficients and partial correlations WRIGHT (1932) reanalyzed the data on the rabbit given by CASTLE (1914 and 1922), and, in addition, that given by DUNN (1928) on the white Leghorn fowl. WRIGHT concludes that, "In all of these (except the F_1 rabbits) the influence of general size factors preponderates, but the residuals indicate the existence of group factors for

the head apart from general size, of group factors for the fore-limbs and hind-limbs collectively, for the hind-limbs separately and for the wings in fowls, the only case in which two fore-limb measurements were available. Special factors acting on each part separately from the others are also indicated. The genetic differences in the rabbit case were largely in general size, but to a small extent in group and special factors."

The researches of SUMNER (1923) and GREEN (1931) seem to indicate that in the mouse both general and specific factors may affect size. It should be emphasized that in neither the mouse nor the rabbit has there been any selection for differences in types of conformation such as is found in the "dairy" and "beef" types of cattle. It seems reasonable to expect where specific growth factors, which affect muscle diameter (volume), are present they would demonstrate themselves less clearly in species which lack marked contrasting differences in body conformation than in species which have distinct and marked contrasting types of conformation, such as are found in the highly specialized "beef" and "dairy" breeds of cattle, especially since these have been reasonably well purified genetically by selection. For this reason it seems that if breeding tests are not desired, this fundamental question concerning the general or specific nature of growth factors can be studied with profit and advantage in cattle or other domestic animals which present contrasting types of conformation.

In mice, guinea-pigs, rats, rabbits and domestic fowls, it has been clearly demonstrated that size is a quantitative character. Since environmental agencies complicate the problem, all studies in size inheritance in mammals have been somewhat disappointing, especially since no clear-cut size genes have been demonstrated with complete satisfaction. This paper is concerned chiefly with quantitative inheritance. For this reason mutant genes such as dwarfness, which are discontinuous in their effect, will not be considered, as in such cases segregation is clear cut and does not follow laws of quantitative inheritance. Moreover, it should be emphasized that this study makes no attempt to determine whether the factors which control skeletal development are general, group or specific, or a combination of the three. However, from WRIGHT'S analysis one should expect the operation of all three with the influence of general factors preponderating.

In a study of growing dairy heifers, ECKLES and SWETT (1919) found that almost any unknown important skeletal measurement can be calculated with a high degree of accuracy from one known skeletal measurement. They also found that height at withers alone was a satisfactory criterion of skeletal development so far as their purpose was concerned. From their investigations it appears that the factors controlling linear

skeletal development in cattle are chiefly general in their action. It should be understood, however, that their studies are not sufficiently extensive to demonstrate the action of group or specific factors which affect skeletal development even if any are involved.

The paper of SWETT, GRAVES and MILLER (1929) which gives an intensive comparative study of the differences in conformation between a highly specialized beef cow and a highly specialized dairy cow, should be mentioned. These investigators came to the conclusion that the skeletal development of the two cows was generally similar, and that the great difference in conformation between the two was caused by the development of extreme "fleshing" on the part of the Angus and the lack of extreme "fleshing" on the part of the Jersey. Unfortunately, the term "fleshing" is not specific. As we understand it the term includes both muscle and fat.

If the problem of size inheritance and conformation is to be solved satisfactorily, it seems that the animal so far as possible should be divided into its component parts of skeleton, muscle, and fat and the inheritance of each part attacked separately. Obviously, many difficulties present themselves in this method. Yet it is a more refined procedure than considering the whole animal as a unit, especially if the investigator can measure muscle and skeletal units with a reliable degree of accuracy.

From the analysis of the development of muscle diameter evidence will be presented in this paper which indicates that some of the genetic agencies which control muscle development are general in their influence. This study is not designed to measure and evaluate the effect of group and specific factors even if some are involved.

Evidence will also be presented which indicates that the genetic agencies affecting linear skeletal development in cattle are different in nature and exhibit a certain degree of independence from those affecting the development of muscle diameter. This indicates that the genetic constitution which affects size and general conformation of an animal may be subdivided, partially at least, into two groups so far as specificity is concerned. One group of genetic agencies is specific for linear skeletal development while the other is specific for the development of muscle diameter. It should be understood that muscles fit the bones so far as linear development is concerned. However, muscles may have a large or small diameter which would, as a consequence, affect the volume (weight). The evidence supporting the preceding statement forms the major part of this study. This does not preclude the possibility that some factors affecting development may influence both muscle diameter and the linear skeleton.

MATERIAL

The Experiment Station herd of the University of California furnished material for this study. The beef breeds used were Hereford, Shorthorn and Angus. In some cases the F_1 hybrids between these breeds were also included. The dairy breeds were represented by Jerseys, Guernseys, Holsteins and Ayrshires. The Hereford and Angus breeds are similar morphologically, since they are of about the same height and weight, and have about the same muscle measurements. The Shorthorns are slightly taller and heavier than either of the other two beef breeds studied. Of the dairy breeds, the Jerseys and Guernseys are almost identical in build and, as a consequence, their heights, weights, and muscle measurements fall into the same ranges. The Holsteins are distinctly taller than the other dairy breeds. This difference in height is discontinuous with the other breeds. The Ayrshires vary greatly in height at withers (skeletal development), muscle development and, consequently, in weight. There is probably more genetic variability in the Ayrshire breed than in any of the other breeds used in this study.

Approximately three hundred animals furnished data for this study. Of these the greater part were females, but in some of the younger age groups a few steers and bulls were used in the analysis of conformation. In all cases where males were used in the analysis with females, morphological differences in conformation between the sexes caused by the male sex hormone had not become apparent from casual observation.

ENVIRONMENT AND FEEDING

The growing dairy calves used in this study were not maintained under the same nutritional conditions as the beef calves. Mead (1929) described the method used in feeding the growing dairy calves. In brief it is as follows: During the first two weeks of their life, the calves were fed whole milk. This was gradually replaced by skimmed milk, the amount not to exceed eighteen pounds daily per calf. After they were from ten days to two weeks old the calves were given a concentrate mixture in addition to the skimmed milk and all the alfalfa hay they would consume. The beef calves, on the other hand, remained with their dams until they were about seven months old. After a few weeks of age, they were given alfalfa hay and concentrates. During part of the year the beef calves were allowed to run on pasture. The greatest difference in the two methods was that the beef calves were allowed to remain with their dams, and even provided with a nurse cow in cases where the dam was a poor producer, while the dairy calves were taken from their dams soon after birth and given skimmed milk. After the dairy and beef calves were weaned their treatments were more nearly identical. When good pasture was available no other food was

given to either group. When they did not have access to good pasture they were supplied with hay and concentrates in amounts sufficient to maintain them in good condition.

The dairy cows which tended to lose weight on account of heavy or excessive lactation were fed in such a manner as would enable them to maintain a relatively constant body weight. Lactation in the beef breeds did not change the weight of the dam to any great extent. In collecting the data no correction was made for variation in weight caused by pregnancy. However, the relatively slight errors from this source do not affect the general trend of the data. No corrections were made for variation in weight caused by condition or amount of fat, although this might affect weight to a slight degree. All the cattle used in the study were breeding cows or growing animals of both sexes kept in a relatively uniform condition. Those fitted for either the show ring or the block were not included.

METHODS

In this investigation a criterion of linear skeletal development and a criterion of muscle development were established. These were then correlated with each other and with the total body weight. Since the age of the animal is an important factor to be considered, all comparisons of height and muscle development between breeds must be made with individuals of the same age or within limited age groups. For this reason in most all of the comparative analyses between breeds mature cows were used. Since both skeletal and muscle development are taking place simultaneously in young rapidly growing animals, it is extremely difficult to distinguish entirely the effect of skeletal increase from muscle increase on total body weight. However, this may be accomplished if the age limit is exceedingly narrow and the numbers compared are relatively large, in order to eliminate unavoidable environmental variations. Since the number of immature animals was limited, an extensive comparative study of height and muscle between the different breeds for the younger age groups was not undertaken.

In this study the general conformation of each animal is expressed in statistical units. This was accomplished by using the muscle measurement as a numerator and height at withers as the denominator. The mathematical index thus obtained represents in a general way the ratio of muscle diameter to linear skeleton. Different breeds were then analyzed as to conformation by means of this muscle-skeleton ratio, which, from now on, will be referred to as the "muscle-skeletal" index or ratio. Since the muscle-skeletal index becomes practically constant relatively early in the life of the animals, and is independent of the influence of age on weight, it is a

reliable method for expressing differences in conformation between individuals and between breeds. In fact, by using this method of analysis all individuals measured, regardless of age, could be used in comparative studies of breed differences.

a. Height at withers

As has been stated before, the criterion for skeletal development is height at withers. For the sake of brevity, height at withers will be expressed by the term "height." It should be remembered that ECKLES and SWETT found height a satisfactory method of expressing skeletal development and that in the living animal almost any unknown linear skeletal measurement could be calculated with a high degree of accuracy from this one skeletal length. This measurement is a standard method for expressing skeletal development and, if care is taken, can be made with only a slight error.

b. The round measurement

On account of the inability to locate definite fixed points it is difficult to obtain accurate non-skeletal body measurements. If no fixed points are used the measurements may vary greatly from time to time and investigators may not be able to duplicate their own or the measurements of others. In order to obtain data as accurate as possible, the muscles in the region of the round were selected for measurement, and two fixed skeletal points were chosen from which to make the measurement. A tape, graduated in centimeters, was placed on the anterior external point of one patella, the first fixed point, and then drawn posteriorly and horizontally about the muscles of the round to a corresponding point on the opposite patella, the second fixed point. The length thus obtained from patella to patella was used as a criterion for the development of muscle diameter. Figure 1 illustrates the method of taking the muscle measurement.

Since the measurement described above has never been used before, some consideration should be given to its appropriateness as a criterion for a muscle measurement. TROWBRIDGE, MOULTON and HAIGH (1918) showed that of all the cuts from the carcass of a beef, the round has the highest percentage of lean (muscle) and that this percentage of muscle to bone and fat remains relatively constant (approximately 80 percent) under the nutritional conditions of maintenance, sub-maintenance and supermaintenance. The fact that the percentage of muscle to bone and fat in the round remains relatively constant even when animals are on different planes of nutrition, together with the fact that the round measurement can be obtained from two definitely fixed points, makes the measurement particularly well adapted as a means of determining the relative amount of muscle in the living animal.

At this point we should give some consideration to the accuracy of the round measurement. In obtaining the measurement, the animal was placed on a level surface, usually a scale, and caused to assume a "normal" standing position, the hind legs being neither too far apart nor too close together, and the hind feet being parallel to a transverse plane through the body. Cow H 154 was measured one hundred times in succession. After

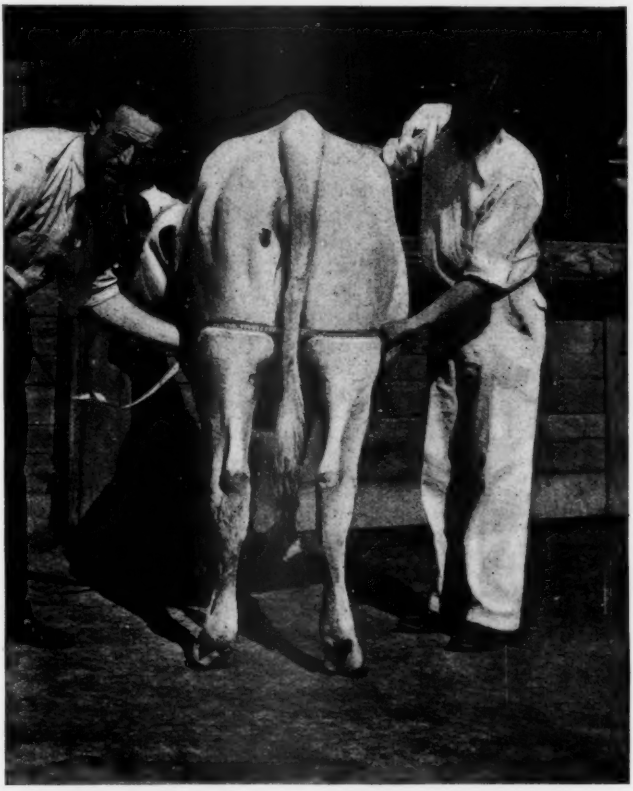

FIGURE 1.—The method used in taking the round measurement.

every third measurement she was forced to change her position, and no attempt was made to have her assume a "normal" position, except that her feet were placed approximately parallel to a transverse plane through the body. They might assume a position close together or far apart, just as she chanced to place herself. The measurements of her round ranged from 93 to 101 centimeters with a mean of $96.4 \pm .139$ centimeters. The standard

deviation was 2.07 centimeters. Table 1 gives a summary of the measurements taken while she was standing in chance positions. The round of this

TABLE 1

Cow H 154, one hundred successive measurements of the round at different positions.

	f	$f.v$	d	d^2	fd^2
93	4	372	−3.4	11.56	46.24
94	16	1504	−2.4	5.76	92.16
95	19	1805	−1.4	1.96	37.24
96	20	1920	−0.4	.16	3.20
97	13	1261	0.6	.36	4.68
98	8	784	1.6	2.56	20.48
99	8	792	2.6	6.76	54.03
100	10	1000	3.6	12.96	129.6
101	2	202	4.6	21.16	42.32
	100	9640			430.00

M 96.40 ± .139 S.D. 2.073

same cow H 154 was next measured fifty-seven times in succession after she was made to assume the normal position described above. She was compelled to shift position after every third measurement, but was always forced to assume a "normal" position. The results of this series of measurements are given in table 2. The range is from 94 to 99 centimeters with a mean of 96.1 ± .097, and a standard deviation of 1.086. The general practice was to take the average of three successive measurements of the round of the animal as the correct one, the animal being made to shift her position between two of the measurements at least. Since in the collection of these data extreme precautions were taken in order to obtain accurate measurements, the actual errors are probably slightly smaller than the one given in table 2.

TABLE 2

Cow H 154, fifty-seven successive measurements of the round at "normal" positions.

	f	$f.v$	d	d^2	fd^2
94	3	282	−2.1	4.41	13.23
95	14	1330	−1.1	1.21	16.94
96	21	2016	−0.1	.01	.21
97	13	1261	0.9	.81	10.53
98	5	490	1.9	3.61	18.05
99	1	99	2.9	8.41	8.41
	57	5478			67.37

Mean 96.1 ± .097 S.D. 1.086

HEIGHT IN MATURE ANIMALS

Since this is a comparative study in the various breeds of the differences caused by genetic factors for growth, an analysis of height and its relation to total body weight was made only for mature animals over four years of age. It should be remembered that ECKLES and SWETT (1918) and ECKLES (1920) showed that by the time Jerseys are four years of age they have reached their maximum skeletal development, and after this age they increase slightly in weight only. According to ECKLES, Ayrshires in their skeletal development follow the Jerseys closely, but are heavier in weight. On the other hand, Holsteins and dairy Shorthorns do not complete their skeletal development until they are almost five years of age, and probably do not reach their maximum body weight until about two years after skeletal development ceases. Although a few of the animals included in this mature class of four years or over have not reached their maximum weight and still fewer have possibly not reached their maximum skeletal development, the slight error from this source does not materially affect the data for mature cows nor the conclusions drawn from them.

The heights of all animals four years or more of age are summarized in table 3. On account of their morphological similarity, the Angus cows are summarized with the Herefords, and the Guernseys with the Jerseys.

TABLE 3

Height in centimeters of mature cows of all breeds.[1]

BREEDS	119 118–120	122 121–123	125 124–126	128 127–129	131 130–132	134 133–135	137 136–138	140 139–141	TOTAL	MEAN	S.D.
Hereford	3	3	4						13	122.96±.488	2.61
Angus	1	1	1								
Jersey		3	16	16	1				39	126.91±.211	1.96
Guernsey		1		1	1						
Ayrshire			3	2	2				7	127.92±.622	2.44
Shorthorn			5	3	3	2			13	128.96±.615	3.29
Holstein						2	5	2	9	137.83±.366	1.63

[1] The original data from which all tables, correlation coefficients, and graphs were made were too massive to include in this paper. The original data are on file at the Brooklyn Botanic Garden and are available on request to any one who wishes to see them.

If height at withers is taken as the criterion for linear skeletal development the Hereford-Angus is less developed than the Jersey-Guernsey group, their height ranging from 118 to 126 centimeters with a mean of 122.96±.488 standard deviation 2.61. The Jersey-Guernsey group is distinctly higher with a range in height from 121 to 130 centimeters, a mean

of 126.91 ± .211, and a standard deviation of 1.96. The limited number of Ayrshires show a mean height of 127.92. The Shorthorns have a range of 125 to 134 centimeters with a mean of 128.96 ± .615, and standard deviation of 3.29. By this they show considerable variability. The limited number of Holsteins are discontinuously taller than any of the other breeds, since they have a range of 135 to 140 centimeters and a mean of 137.83. Although the total number of mature animals is eighty-one, the numbers are comparatively small when the animals are subdivided and grouped according to breeds. Yet it seems safe to assume that the height found to be the mean for each breed, represents approximately the genetic capacity of the heights of the breeds of the University of California.

With height at withers as a criterion of linear skeletal development, it is evident that there are several different genetic constitutions for height (linear skeletal development) in the different breeds studied. When the Hereford-Angus group is compared with the Jersey-Guernsey group the difference between the mean heights is 3.95 ± .531. This difference is over seven times the probable error of the difference. When the mean height of the Jersey-Guernsey group is compared with the Shorthorns the difference between the mean is 2.05 ± .65. This difference is only three times the probable error and is of doubtful significance. However, if larger numbers were observed the difference in height might be found to be significant. The height of the Holsteins is discontinuously greater than that of the Shorthorns. The difference between the means is 8.87 ± .715. This is more than twelve times the probable error. From this analysis of height it is evident that there are at least three different genetic capacities for height, with some indication of a fourth. The Hereford-Angus group, which has the least linear skeletal development, composes one group. The Jersey-Guernsey group, which is second in linear skeletal development, comprises the second group, while the Holsteins compose the third group. If there is a fourth genetic constitution for height, it is obviously made up of Shorthorns. The differences in height of all breeds, except Holsteins, are of a continuous nature, while that of the Holsteins is clearly discontinuous.

ROUND MEASUREMENT IN MATURE ANIMALS

The mature animals of all breeds which were analyzed for height (table 3) were analyzed for round measurements (table 4). For the Jersey-Guernsey group, the round measurement ranged from 83 to 100 centimeters with a mean of 90.75 ± .351, standard deviation 3.25. The Jersey cow which falls in the 100 centimeter class should probably be eliminated since she is a nymphomaniac. (Four other nymphomaniacs were included in the Jersey group. All four were at the upper limit of the range in round measurement as well as body weight.) The round measurements of the

TABLE 4

Round measurement in centimeters of mature cows of all breeds.

BREED	84 83–85	87 86–88	90 89–91	93 92–94	96 95–97	99 98–100	102 101–103	105 104–106	108 107–109	111 110–112	114 113–115	TOTAL	MEAN	S.D.
Jersey	5	11	8	7	4	1						39	90.75±.351	3.25
Guernsey	1	1		1										
Ayrshire			1		1	1	2	1	1			7	100.92±1.41	5.55
Holstein						1	3	5				9	103.94±.472	2.10
Shorthorn						1	3	4	3	2		13	106.11±.657	3.52
Hereford						2		4	2	1	1	13	106.75±.755	4.04
Angus								1	2					

Shorthorns range from 99 to 112 centimeters with a mean of 106.11 ± .657, standard deviation 3.52. The Hereford-Angus group ranges from 100 to 115 centimeters with a mean of 106.75 ± .755, standard deviation 4.04. The number of Holsteins is slightly more limited than some of the other groups, the range being from 99 to 106 centimeters, with a mean of 103.94.

When compared with the Jersey-Guernsey group on the one hand, and the beef breeds on the other, the Ayrshires present some very interesting data. It should be recalled that this group is near the same height as the other breeds (Holsteins excepted) but is intermediate in round measurement, the range being from 90 to 107 centimeters. The Ayrshire is a dairy breed, but some strains have been selected along dual purpose lines. Some consideration should be given to the number of different genetic capacities for round measurement. When the mean round measurement of the Jersey-Guernsey group is compared with that of the Holsteins the difference is 13.19 ± .588. This difference is 22 times the probable error. The comparison of round measurement of the Jersey-Guernsey group is not made with either of the beef groups since it is clearly evident that the difference is significant without this analysis. When the mean round measurement of the Holsteins is compared with that of the Hereford-Angus group the difference is 2.81 ± .915. This difference is only three times the probable error and is of doubtful significance.

The next question for consideration is how many different genetic complexes are there for round measurement or the development of muscle diameter? There are unmistakably two, and possibly more—one for the Jersey-Guernsey group and one for the beef breeds (Hereford, Angus and Shorthorns). In round measurement the Holsteins are somewhat intermediate between the Jersey-Guernsey group on the one hand and the beef breeds on the other, but on account of their great discontinuity for height it is difficult to interpret and evaluate the significance of their round measurement. The Ayrshire breed indicates that some segregation is oc-

curring so far as muscle diameter is concerned. This is indicated by the variability of the round measurement. In muscle development they are intermediate between beef and dairy breeds and tend to range over the whole series. This appears to agree with the observations of KUHLMAN (1915) in crosses between Jersey and Angus cattle. F_1 hybrids of this cross were somewhat intermediate, but were nearer the Angus or beef type of conformation, while the F_2 and subsequent generations showed greater variability in types of conformation. It is unfortunate that a larger number of the Ayrshires was not available for analysis.

WEIGHT IN MATURE ANIMALS

On account of numerous environmental factors which one cannot control, it is exceedingly difficult to standardize weight in mature cows. Most of this variability is caused by change in condition such as varying amounts of fat, pregnancy and, in dairy cows, lactation.

In their investigations at the Missouri station, TROWBRIDGE, MOULTON and HAIGH (1918, 1921, 1922, 1923) showed that in Hereford-Shorthorn cattle, the greatest increase in fatty tissue takes place in the plate, loin and flank. An increase also takes place in the rib and rump, but to a slightly less degree. Now, since only a slight amount of fat is deposited in the region of the round, appreciable changes in body weight caused by variation in amount of body fat, can take place without causing a corresponding change in the round. This fact is demonstrated by Hereford cow number 199. On September 16, 1930, her round measurement was 105 centimeters and her weight was 1290 pounds. On February 24, 1931, after an illness, caused by a uterine infection, she had lost 315 pounds, but retained a round measurement of 104 centimeters—a loss of only 1 centimeter. In other words, she suffered a considerable change in body weight which was unquestionably due to loss of fat, but her round measurement remained within the limit of the error of measurement. This instance gives additional evidence that the round measurement is primarily a measurement of muscle diameter.

In table 5 are summarized the weights of all the mature cows shown for height in table 3 and round measurement in table 4. The Jersey-Guernsey group ranges from the class value of 924 to 1324 pounds with a mean of 1076 ± 11, standard deviation 103. The Hereford-Angus group ranges from 1074 to 1574 pounds with a mean of 1334 ± 27, and a standard deviation of 146. The Shorthorns range from 1174 to 1724 pounds. They have a mean of 1432 ± 26, and a standard deviation of 139. The Holsteins range from 1224 to 1524 pounds with a mean of 1375. In this analysis of weight, the Ayrshires tend to be intermediate between the Jersey-Guernsey group and the beef group.

TABLE 5

Weight in pounds of mature cows of all breeds.

BREED	924–974	1024–1074	1124–1174	1224–1274	1324–1374	1424–1474	1524–1574	1724–1824	TOTAL	MEAN	S.D.
Jersey	10	11	11	3	1				39	1076.3±11.1	103.16
Guernsey	1	1	1								
Ayrshire	1		1	3	1	1			7	1239.2±35.8	140.69
Hereford		1	1	2		5	1		13	1334. ±27.3	146.15
Angus			1			2					
Holstein				2	4	2	1		9	1375. ±19.4	86.6
Shorthorn			1	1	3	4	3	1	13	1432.6±26.1	139.8

RELATION OF HEIGHT, MUSCLE DEVELOPMENT AND WEIGHT IN MATURE ANIMALS OF DIFFERENT BREEDS

CASTLE (1914 and 1922) found in the rabbit that for a given linear skeletal length there is a proportional amount of development of other tissues. Evidently, linear skeletal measurements and muscle diameter are

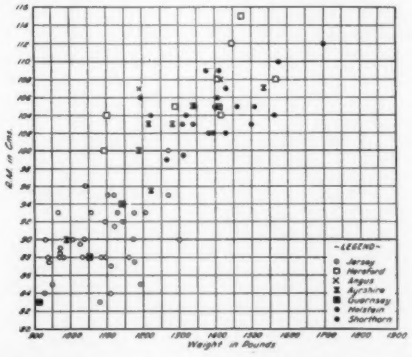

FIGURE 2.—Round measurement plotted against weight for mature cows of all breeds.

highly correlated with each other and both are correlated to about the same degree with total body weight. Now, if the same condition prevails in cattle these correlation coefficients should also prevail. The correlation coefficients have been determined for the mature cows shown in tables 3, 4 and 5 and the results, which are exceedingly interesting, are shown in table 6. Since the Holsteins are discontinuous with all the other breeds so far as linear skeletal development is concerned, correlation coefficients were determined both with and without the Holsteins.

When all breeds are considered together, the correlation coefficient of round measurement, the criterion of muscle development, with weight is

$+.875 \pm .017$. When the Holsteins are omitted only the breeds which are generally similar in linear skeletal development remain. The correlation coefficient of round measurement with weight is $+.896 \pm .015$. This is slightly higher than when the Holsteins are included. It is clearly evident from this that muscle development is highly correlated with body weight.

When all breeds are considered together, the correlation coefficient of height, the criterion of linear skeletal development, with weight is $+.383 \pm .064$. If the Holsteins are omitted the correlation coefficient is $+.040 \pm .079$. The evidence indicates that in mature cows linear skeletal development with associated muscle lengths is not the most important factor affecting weight. Furthermore, it indicates that some of the genetic

TABLE 6
Correlation coefficients of mature cows.

	TOTAL	CORRELATION COEFFICIENT	
R. M. with weight (all breeds)	80	+.875	±.017
R. M. with weight (Holsteins omitted)	71	+.896	±.015
R. M. with weight (Mature cows all of the same height class of 125–127 centimeters)	30	+.889	±.021
Height with weight (all breeds)	80	+.383	±.064
Height with weight (Holsteins omitted)	71	+.040	±.079
R. M. with height (all breeds)	80	+.190	±.072
R. M. with height (Holsteins omitted)	71	−.016	±.08

agencies which control muscle diameter differ from and are possibly independent of (not linked with) those which control linear skeletal development. A more critical test of this is made when round measurement (the

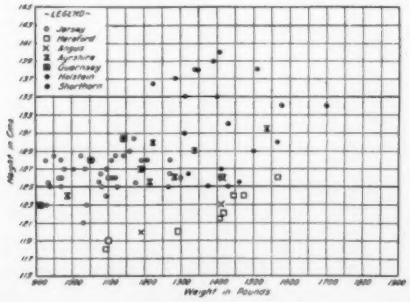

FIGURE 3.—Height plotted against weight for mature cows of all breeds.

criterion of muscle development) is correlated with height at withers, the index of linear skeletal development. When the round measurements of mature cows of all breeds are correlated with their heights the correlation coefficient is $+.190 \pm .072$. When the Holsteins are omitted this correlation

coefficient drops to $-.016 \pm .08$. A still more critical test to determine the dependence or independence of the genetic agencies which affect muscle diameter and linear skeletal development was made by eliminating the effect of linear skeletal development. This was accomplished by taking all mature animals in the height at withers class of 125 to 127 centimeters and correlating their round measurements with their weights. Obviously all Holsteins were omitted. The correlation coefficient for the thirty animals tested in this manner was found to be $+.899 \pm .021$, which is almost as high as $+.896 \pm .015$ the correlation coefficient of round measurement with weight when the Holsteins were omitted.

The evidence obtained from the analysis by means of correlations appears to be conclusive proof that some of the genetic agencies which control the development of muscle diameter in mature animals differ from and are possibly, though not necessarily, genetically independent of those which control linear skeletal development.

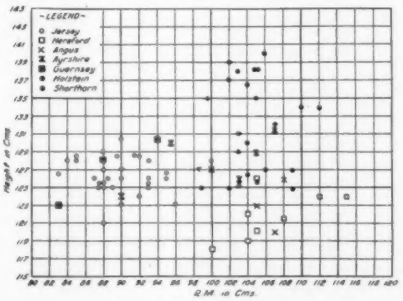

FIGURE 4.—Height plotted against round measurement for mature cows of all breeds.

The data concerning the immature animals as a whole are numerous, but when restricted to narrow limited age groups, they are not numerous enough to analyze for any particular age class when the relations of muscle development and total body weight are considered. For this reason another method of analysis is used for determining the nature of growth factors in animals of all ages.

RELATION OF HEIGHT, MUSCLE DEVELOPMENT AND FAT TO WEIGHT IN NEWLY-BORN CALVES OF DAIRY AND BEEF BREEDS

Some consideration should be given to the development of muscle, skeleton, and fat in relation to total body weight in newly-born calves of different breeds. Although we have no large mass of data bearing on this subject, what we have is sufficient to establish certain important facts. The calves included in this analysis range from one day to sixteen days of age.

The majority, however, is seven days or less. The age class range should have narrower limits. The data are summarized in table 7.

TABLE 7

Height, weight, and round measurement in newly-born calves of different breeds.

			AGE IN DAYS	HEIGHT	ROUND MEASUREMENT	WEIGHT
Jersey	Female	512	13	65.5	39	52
	"	513	4	64.5	37.5	45
	"	514	5	65.5	40	47
	Male	58E	9	61.0	44	55
	"	57E	10	68.0	44	63
	Female	520	4	70	37	53
	Male	(Dam 426)	3	63.5	40.5	52
	"	(Dam 402)	2	68.5	41.0	63
	"	(Dam 444)	2	69.0	40.0	52
	Female	521	1	58	33.5	34
	"	522	1	65.5	41	54
Guernsey	Male	607A	1	69	44.5	64
Mean				65.6	40.1	52.8
Hereford	Female	436	8	67.5	55	100
	"	437	7	70	58	
	"	448	7	65.5	46	67
	"	449	4	57.5	39.5	45
	"	450	16	65.0	53	82
Angus	"	434	7	65.0	53	90
Hereford	"	446	6	65.0	51.0	75
Angus	"	432	16	65.5	54.0	100
	"	433	16	68.0	55.0	115
	"	482		64.5	42	48
Shorthorn	"	445	11	65.0	47	75
Twins	Male	463	14	65.0	45	75
	Male	464	14	66	47.5	80
Mean				65.3	49.7	79.3
Holstein	Female	183	9	72	51	90
	"	184	3	71	50.5	86
	"	187	2	68	45	61
	"	188	5	72	52	84
	"	189	1	71.5	46.5	81
Mean				70.9	49	80.4
Ayrshire	Female	249	7	71.5	53	82
	"	250	7	69	50.5	85

The range in height of the Jersey-Guernsey group is from 58 to 70 centimeters with a mean of 65.6. The range in round measurement is from

33.5 to 44.5 centimeters with a mean of 40.1. The weight ranges from 34 to 64 pounds with a mean of 52.8.

The range in height for the Hereford-Angus-Shorthorn group is from 57.5 to 68 centimeters with a mean of 65.3. The round measurement varies from 39.5 to 58 centimeters with a mean of 49.7. The range in weight is from 45 to 115 pounds with a mean of 79.3. Since the number of Holsteins and Ayrshires is limited, no attempt has been made to analyze the results. However, the weight of the two Ayrshire calves is slightly higher than the mean birth weight of 72 pounds as given by ECKLES. The Holstein calves appear on the other hand, to weigh slightly less than the ones reported by him. The mean weight of the Jerseys, 52.8 pounds, closely approximates the mean birth weight of Jerseys reported by ECKLES (1919) for the Missouri herd.

The mean weight of the newly-born Hereford-Angus-Shorthorn calves, is 79.3 which is close to that given by HAIGH, MOULTON and TROWBRIDGE (1920). They reported that four calves from dams which were on a high plane of nutrition averaged 82.53 pounds, while five calves from dams on a medium plane of nutrition averaged 80.87 pounds.

When the height of the Jersey-Guernsey group is compared with that of the beef group, it slightly exceeds the latter, though the difference is insignificant. On the other hand, when the mean round measurement and weight of the Jersey-Guernsey group are compared with those of the beef group, the beef group unmistakably exceeds in both of these measurements. Here again we find that weight is related to round measurement (muscle diameter).

Is the difference in weight between the newly-born Jersey-Guernsey calves on one hand, and the beef calves on the other, caused by differences in muscle development or in the amount of fat? The investigations of HAIGH, MOULTON and TROWBRIDGE (1920) on the composition of the bovine at birth, give some information concerning this question. Jersey calves 11-A and 11-B contained 4.591 and 3.494 percent of fat respectively in the total carcass. Four Hereford calves on a high plane of nutrition contained from 3.8 to 4.4 percent fat with a mean of 4.155 in the entire carcass. Five Hereford calves on a medium plane of nutrition had a range of 3.2 to 3.95 percent of fat in the entire carcass. The mean was 3.568. From the study at the Missouri station, it is evident that differences in total fat of the newly-born calves of dairy and beef breeds will not account for differences in birth weight, but differences in development of muscle diameter will account for a large part, if not all, of the difference in birth weights.

GENERAL CONFORMATION

a. Mature cows

Since ECKLES, and ECKLES and SWETT, showed that almost any linear skeletal measurement may be calculated with a high degree of accuracy from the measurement of the height at withers alone, it is evident that in cattle the factors which control linear skeletal development are chiefly, if not exclusively, general in their effect. This also seems to agree with WRIGHT'S analysis for skeletal development for rabbits and White Leghorn fowls. The question then arises as to whether or not the agencies which control the development of muscle diameter are chiefly general with group and specific factors having a minor effect. WRIGHT'S methods of analysis should definitely settle this question. However, since it was not practical to make such an analysis, a more indirect method was used.

In the mature animals, the correlation coefficients of round measurement with weight was very high as shown in the previous discussion and in table 6. The three correlation coefficients of round measurement with weight were $+.875, \pm.017$; $+.896, \pm.015$; and $+.889 \pm .021$, depending upon the grouping of the animals used in the study. From these it is evident that some of the agencies which control the development of muscle diameter have a general effect and indicate that many muscles were influenced. Furthermore, if specific factors which affect individual muscles or groups of muscles are involved, they evidently play a subordinate role in their total effect on body weight. For this reason, from the standpoint of expressing the build or general conformation (relation of muscle diameter to linear skeletal development) of an animal one may disregard the possible effect of group or specific growth factors on the development of muscle diameter as well as on linear skeletal development, though it is highly probable that some are involved in both systems.

By taking the round measurement as the numerator and the linear skeletal measurement of height at withers as the denominator (R.M./H) one may express general conformation in statistical units. This expression gives an abstract number which represents in a general way the ratio of muscle diameter to linear skeletal development, and which is diagnostic of build or general conformation. This standard of the measurement of conformation is referred to in this paper as the muscle-skeletal index or ratio. Table 8 shows the summary of the muscle-skeletal index of mature cows of all breeds studied. Since all of these numbers are decimals, it may be more convenient to express the index as whole numbers by omitting the decimal point, but in this analysis the decimal point is retained though in table 8 the decimal point is omitted and the index is carried to two figures only.

TABLE 8
Index of mature cows of all breeds.

BREED	GROUP NO.	RANGE IN INDEX	TOTAL	MEAN	S.D.	GROUPS COMPARED	MEAN DIFFERENCES	DIF. / P. E.
Jersey Guernsey	1	65–77 66–72	39	71±.3	3.1	1–2	4.4±.46	9.5
Holstein	2	72–78	9	75±.3	1.4	2–4	6.9±.55	12.4
Ayrshire	3	72–82	7	79±.9	3.7			
Shorthorn	4	78–87	13	82±.4	2.4	4–5	5.0±.65	7.7
Hereford Angus	5	82–93 84–89	13	87±.5	2.5			

The Jersey-Guernsey group has a range in the muscle-skeletal index from .6595 to .7795 with a mean of .709±.003, and standard deviation of .031. The Holsteins range from .7295 to .7795 with a mean of .753±.003, standard deviation .014. Although limited in number, the Ayrshires show two trends of type of conformation, one a dairy type and the other beef. In spite of this, however, the Ayrshires may be classed as somewhat intermediate between the beef and dairy types of conformation. They range from .7295 to .8195 with a mean of .787±.009, standard deviation .037. The muscle-skeleton index of the Shorthorns ranges from .7895 to .8695 with a mean of .822±.004, standard deviation .024. The Hereford-Angus group ranges from .8295 to .9295 with a mean of .872±.004, standard deviation .025.

Since Holsteins are discontinuous with the other breeds for skeletal development, and since the Ayrshires are somewhat intermediate in conformation between the beef and dairy types, we shall compare the Jerseys and Guernseys with the combined beef breeds, Shorthorn, Hereford, and Angus. The mean muscle-skeletal index for the Jersey-Guernsey group is .709±.003, while it is .847±.001 for the combined beef breeds. When the probable error of the difference is considered, we find that the deviation is more than thirty-eight times the probable error of the difference.

From this analysis it is evident that mature cows of the various breeds, or even individual animals which differ in conformation (the ratio of muscle to skeleton) may be separated by the muscle-skeleton index. Expressing the difference in conformation in statistical units on the basis of the muscle-skeletal index is a new method of attack and it may aid in the solution of the complex problem of the inheritance of conformation and size.

b. The muscle-skeleton index in different breeds of all ages

Evidence has been presented which indicates that for mature animals, the muscle-skeleton index is diagnostic of differences in conformation be-

tween the various breeds of cattle, and even between individuals of the same breed when they happen to differ in build. Now the question arises as to whether or not differences in conformation exist throughout the growing periods from birth to maturity. An appreciable amount of data has been gathered which bears on this question. Individuals of all breeds and of all ages from birth to maturity may be used in this analysis if the muscle-skeleton index is plotted against age in months. These data are given in the form of a graph and shown in figure 5. This chart clearly shows several striking facts. The method of expressing conformation in statistical units shows a distinct difference in conformation between beef and dairy breeds from birth to maturity. The figure .785 approximately separates the beef from the strictly dairy breeds. In the foregoing discussion it was stated that so far as dairy and beef types are concerned, the Ayrshire

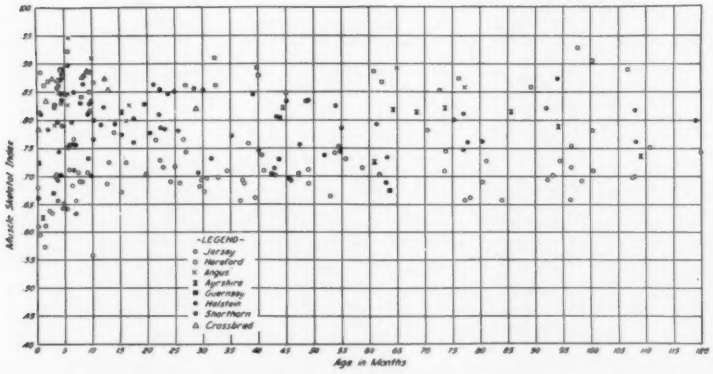

FIGURE 5.—The muscle-skeletal index of animals of all breeds and all ages plotted against age in months.

cows in the Experiment Station herds are of mixed types of conformation. When we examine the analysis in figure 5 this fact is clearly shown. All of the mature Holstein cows fall below the beef breeds in muscle-skeleton index except a few young Holsteins from four to twenty-six months of age, which plot in the lower limit of the range of the beef group.

When the beef breeds are considered together, the Hereford and Angus breeds tend to fall together, and have a muscle-skeleton index slightly higher than the Shorthorns. The beef breeds at birth have a muscle-skeleton index which they tend to retain throughout the growing and adult periods.

The Jerseys and Guernseys plot together and have a low muscle-skeleton index. The Holsteins have a muscle-skeleton index which is slightly higher than that of the Jersey-Guernsey group. The dairy breeds give us a

very interesting picture of conformation from birth to maturity. At birth the ratio of muscle diameter to linear skeletal development for the Jerseys is about .60. This is low. However, the index gradually changes and by the time the Jerseys are eight months of age the muscle-skeletal index has become relatively constant and remains so throughout the growing and adult period. There is an indication that a change in muscle-skeleton index takes place likewise in the Holstein breed, but it is less marked. In other words, the muscle-skeleton index for the Jerseys tends to change from birth

FIGURE 6.—Photograph of mature Jersey cow 421. Height 126 centimeters, round measurement 88.5 centimeters, weight 970 pounds. Note the lack of development of muscle in the round and throughout the body. Muscle-skeletal index 702. Compare with figure 7.

to about eight months of age, whereas that of the beef breeds tends to remain constant from birth to maturity.

A question which deserves consideration concerns the genetic agencies which operate to affect conformation so far as muscle diameter and linear skeletal development are involved. These factors, which affect muscle diameter as found in the breeds studied, are evidently different in nature and are possibly for the most part genetically independent of those affecting linear skeletal development.

There should be as many types of conformation in cattle as it is possible to obtain by uniting all genotypes affecting muscle diameter with all genotypes affecting linear skeletal development.

Relation of the muscle-skeletal ratio to pre-natal and post-natal development

Since the beef breeds start at birth with a muscle-skeletal ratio which remains practically constant throughout life, and since the dairy breeds, especially the Jersey, have a low muscle-skeleton index at birth, but have an increase in amount of muscle in relation to their linear skeletal development for the first eight months, at which time it becomes practically constant and remains so throughout the remainder of the life of the individual, it is clearly evident that the embryological and foetal development of the beef breeds differ in some detail from that of the Jersey breeds. At birth the beef breeds appear to have their adult muscle-skeleton ratio, while it takes the Jerseys several months of post-natal development to reach their adult muscle-skeleton ratio. This seems to indicate that the stimulus which causes the development of muscle tissue is less marked in some dairy breeds during the foetal period than in the beef breeds. This seems to agree in part at least with the condition reported in the rabbit

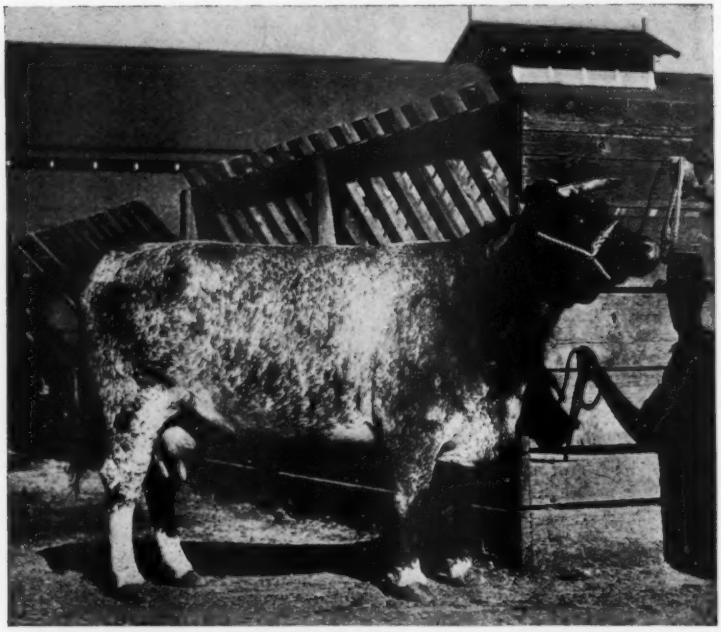

FIGURE 7.—Photograph of mature Shorthorn cow 212. Height 126.5 centimeters, round measurement 104 centimeters, weight 1320 pounds. Note the excessive development of muscle in the round and throughout the body. Muscle-skeletal index 822. Compare with figure 6.

by CASTLE and GREGORY (1929) and GREGORY and CASTLE (1931). They found that the eggs of large races of rabbits segment at a faster rate than those of small races. Now, since some of the genetic agencies which control the development of muscle diameter in cattle are different from and exhibit a certain degree of independence from those which control linear skeletal development, it appears logical to assume that the biochemical substance which causes muscle development is more concentrated in the beef breeds during foetal development than it is in the dairy breeds.

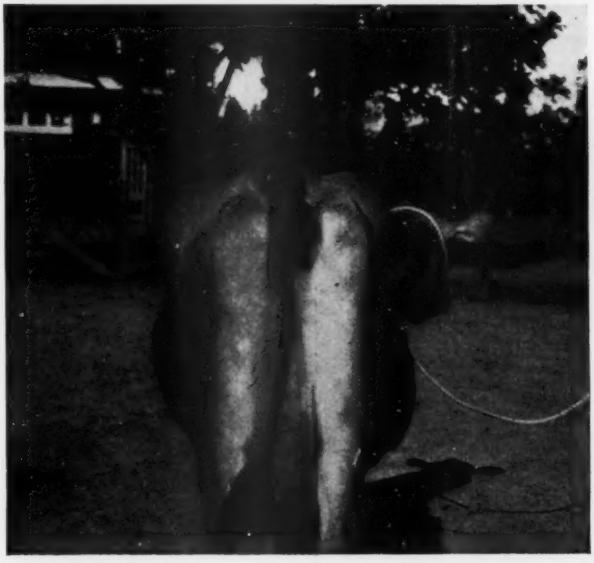

FIGURE 8.—Posterior view of Jersey cow 421. Note the deep depression and lack of muscle development in the round. Compare with figure 9.

GREGORY and GOSS showed that in the rabbit the concentration of sulphydryl is greater in large race rabbits than in small race rabbits. The chemical determinations were based on the analyses of newly-born rabbits which had fasted 48 hours. At this time it is impossible to state the biochemical nature of the substance which stimulates muscle development in cattle, but it may be the concentration of sulphydryl since this biochemical compound is known to stimulate growth by increase in cell number as was shown by HAMMETT and his associates.

DISCUSSION

The nature of growth factors in mammals

The evidence from the data presented in this paper indicates that some of the genetic agencies which control linear skeletal development in domes-

tic breeds of cattle are different from and possibly independent of those which control muscle volume. From an analysis of the data of MACDOWELL and CASTLE (1914), CASTLE (1922) for three populations of rabbits, and from DUNN'S data on the White Leghorn fowl, WRIGHT (1932) seems to have definitely established the existence of three types of growth factors—general, group and specific—which influence skeletal development and weight. General growth factors (with an exception in the F_1 rabbit population) account for most of the total effect, while group and specific factors each contribute a minor part. WRIGHT'S interpretation of growth factors

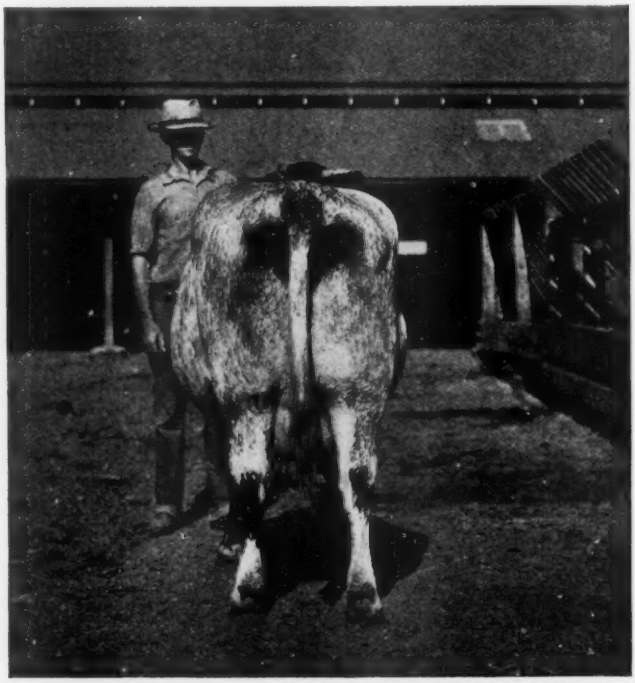

FIGURE 9.—Posterior view of Shorthorn cow 212. Note the lack of depressions and full muscle development of the round. Compare with figure 8.

is in harmony with the observations and conclusions of SUMNER and GREEN for mice.

In several recent publications GREEN gave the results of his work on size inheritance in a mouse species cross between *Mus musculus* and *Mus bactrianus*. In his report on the nature of size factors, he gave the correlation coefficients of various skeletal measurements, most of which were linear, in relation to each other and in relation to total body weight.

This was done for the two species mentioned above, as well as for the F_1 hybrids between the two. From his analysis GREEN came to the conclusion that general and specific factors are both involved in development. The data presented by GREEN seem to indicate that some of the genetic agencies which control linear skeletal development may be independent of some of the genetic agencies which control the development of weight. If this is the case it agrees with the findings in cattle.

In the mouse species cross, GREEN had three chromosomes marked with genes A, B and D. The *musculus* parent carried the recessive genes while the *bactrianus* parent carried their dominant allelomorphs. Since all the brown mice which were produced from F_1 hybrids back-crossed to the *musculus* parent were heavier and had greater bone dimensions (with the exception of skull length and width) than the black mice produced from the same matings, GREEN came to the conclusion that the *musculus* chromosome bearing the brown gene also bore a factor for increased linear skeletal measurements.

GREEN'S analysis does not determine whether or not this growth factor which is borne in the chromosome which bears the brown gene affects other tissues in the body, such as muscle diameter.

Since conformation, the relation of muscle diameter to linear skeletal development, is an important factor in weight and size inheritance, and since differences in conformation seem never to have been taken into account in genetic studies of growth and size inheritance, development of muscle diameter and its effect on weight may be one of the disturbing factors in the interpretation of the nature of growth factors. Since general, group and specific factors influence linear skeletal development, these three types of growth factors may also be involved in influencing muscle diameter and, consequently, volume.

The evidence presented in this study indicates that general factors are responsible for most of the growth in each system (muscle and skeletal), but in neither system does it evaluate their total effect or the influence of group and specific factors. Furthermore, it would be desirable to know if growth factors exist which operate on the development of both muscle diameter and the linear skeleton. In the preceding pages it was shown that Holsteins are discontinuously greater for height at withers (linear skeletal development) and at the same time have an increased round measurement. Can this increased linear skeletal development and increased round measurement be accounted for by a general factor (or factors) which affects both muscle diameter and linear skeletal development, or is size in Holsteins accounted for by factors which increase linear skeletal development combined with factors which cause greater muscle diameter? This study does not answer this fundamental question.

So far as the relation of muscle diameter to linear skeletal development in the beef and dairy animals used in this study is concerned, there are several unmistakably contrasting types of conformation. This in itself is evidence that some of the growth factors which affect development of muscle diameter are different in nature and possibly, though not necessarily independent of (not linked with) those which affect linear skeletal development.

We believe that the muscle-skeleton index may be of value in the analysis of some of the problems of conformation and size inheritance in cattle, and possibly other animals, since it enables one to express general conformation in statistical units. If the factors which influence muscle diameter and linear skeletal development were exclusively general in each system this index, fundamentally at least, should be an almost perfect criterion of conformation. However, it is a practical certainty that group and specific factors are operating to limited extents in both the muscle and the skeletal systems. Therefore, this expression of conformation (relation of muscle diameter to linear skeletal development) is without doubt subject to slight errors which are caused by group and special factors operating in each system. When we determine the exact role that general, group and specific factors play in the development of both muscle diameter and the linear-skeleton, then the absolute value of the muscle-skeleton index may be determined. Whatever the outcome of that study may be, I believe that the preponderance of general growth factors operating in both the muscle and skeletal systems will cause the muscle-skeletal index to have some value as a tool to aid in the analysis of the inheritance of conformation and related phenomena in cattle.

GREEN has shown for mice that the male sex hormone affects the expression of growth factors. He suggested that growth factors are more specialized in males and more general in females. WRIGHT has shown that sex has only a slight effect on growth in rabbits. Since no mature males are used in this study of cattle, we are not certain of the effect of the male sex hormone on conformation. It is evident that the male sex hormone causes a certain amount of modification of conformation in cattle. The magnitude of this modification is yet to be determined.

SUMMARY

1. A study of the nature of growth factors which control muscle diameter and linear skeletal development was made in Hereford, Angus, Shorthorn, Holstein, Jersey, Guernsey and Ayrshire cattle. Most of the animals used were females, but a few males were used in the younger ages.

2. From an analysis by means of correlation coefficients, evidence is presented which indicates that some of the genetic agencies which control

muscle diameter are different in nature and possibly, though not necessarily, genetically independent of the genetic agencies which control linear skeletal development.

3. Evidence is also presented which indicates that some of the factors which control the development of muscle diameter are chiefly general in nature. However, the data do not deny the existence of group and specific factors influencing the development of muscle diameter.

4. There are at least two different genetic compositions which are clearly demonstrated, involved in the development of muscle diameter. It is yet to be demonstrated whether or not other genetic compositions having marked effects are involved.

5. From the study of ECKLES and SWETT on linear skeletal development in cattle the conclusion is reached that the genetic agencies affecting linear skeletal development are chiefly general in nature and if specific factors affecting individual bones, or groups of bones, are involved they play only a minor part in linear skeletal development.

6. The evidence indicates that in the breeds studied, at least three different genetic compositions are involved in height, the criterion of linear skeletal development, and there is some indication of a fourth.

7. Since linear skeletal development in cattle is controlled by growth factors which are chiefly general in nature, and muscle diameter is controlled by growth factors which are also chiefly general in nature, and since genetic agencies which control linear skeletal development are different from and possibly independent of the genetic agencies which control muscular diameter, the general conformation of an animal may be expressed in statistical units by taking the round measurement as the numerator and the height as the denominator $\left(\frac{\text{R. M.}}{\text{H}}\right)$. This represents in a general way the ratio of muscle diameter to linear skeletal development. The conclusion is reached that conformation is primarily the result of the interaction of growth factors (general group and specific) affecting muscle diameter combined with growth factors (general, group and specific) affecting linear skeletal development.

8. Evidence from the study of the muscle-skeleton index also indicates that the genetic agencies which affect the development of muscle diameter have a certain degree of independence from those which affect linear skeletal development.

9. The muscle-skeletal index of the beef breeds studied was practically constant from birth to maturity. In the dairy breeds, especially Jerseys, the muscle skeleton index was not constant from birth to maturity. The evidence indicates that in Jerseys, muscle tissue is retarded during foetal development and the adult muscle-skeletal index is not reached until the

young are about eight months of age. This indicates that muscle development in beef breeds is somewhat more accelerated than in dairy breeds (with the exception of Holsteins) during foetal development, while the rate of linear skeletal development of beef and dairy breeds is about the same.

10. When the nature of growth factors, as reported in mice and rabbits, is considered in relation to the interpretation made for different breeds of cattle, the conclusion is reached that differences in conformation in the races or species studied may account for some of the diversity of opinions held on the general or specific nature of growth factors.

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A CLOSED X CHROMOSOME IN DROSOPHILA MELANOGASTER*

L. V. MORGAN
California Institute of Technology, Pasadena, California

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From a pair of attached X chromosomes of *Drosophila melanogaster* there is derived occasionally a single detached X chromosome which shows all the characteristics of a normal X. In consequence a very small class of regular offspring is found in attached X lines. One yellow female having attached X chromosomes mated to scute broad apricot males produced a regular wild-type female, the phenotype to be expected if an egg bearing a detached X had been fertilized by X sperm; but among the offspring of the wild-type female there was an unexpected class of males. She had been mated to a forked bar male and produced, besides about 12 yellow and 35 scute broad apricot males, 7 forked bar males. The wild-type female had received one yellow-bearing chromosome from her mother and an X chromosome bearing genes for scute broad apricot from her father. If she had received in addition to a single yellow-bearing chromosome also a Y chromosome from her attached X mother, exceptional males would have been expected from Y eggs, but secondary exceptional wild-type females would also have been expected. In fact all of the daughters were heterozygous bar; moreover all of the forked bar males were found to be sterile, and were evidently primary non-disjunctional XO males. Some of the F_1 heterozygous bar females were mated to X-ple males (*sc ec cv ct*^{6} *v g*^{2} *f*) and again sterile non-disjunctional males were produced, and among the regular classes there were unusual ratios (table 1). Whereas about 50 percent of crossing over is expected between yellow and forked, only 6 among 225 regular males showed recombination of the characters.

TABLE 1

Offspring of $\frac{X^{e}y}{fB}$ ♀ × *X-ple* ♂.

NO. OF CULTURE	♀♀		♂♂				
			NON-CROSSOVERS		CROSSOVERS		PATROCLINOUS
	+	*fB*	*y*	*fB*	*y/B*	+	X-ple
2.54a	70	82	56	87	0	3	2
2.54b	166		48	58	0	3	1

* Part of the cost of the accompanying tables is paid from the GALTON AND MENDEL MEMORIAL FUND.

The yellow-bearing chromosome for two generations had shown certain peculiarities which were hereditary. Further tests showed that the chromosome is unique, in that among the offspring of females homozygous or heterozygous for the chromosome there are no single or triple crossovers, but there are many patroclinous males and gynandromorphs are unusually frequent (L. V. MORGAN 1929).

CYTOLOGY

The unique chromosome in preparations of oogonial plates appears to be a thick almost circular ring, clearly hollow in the middle. The ring

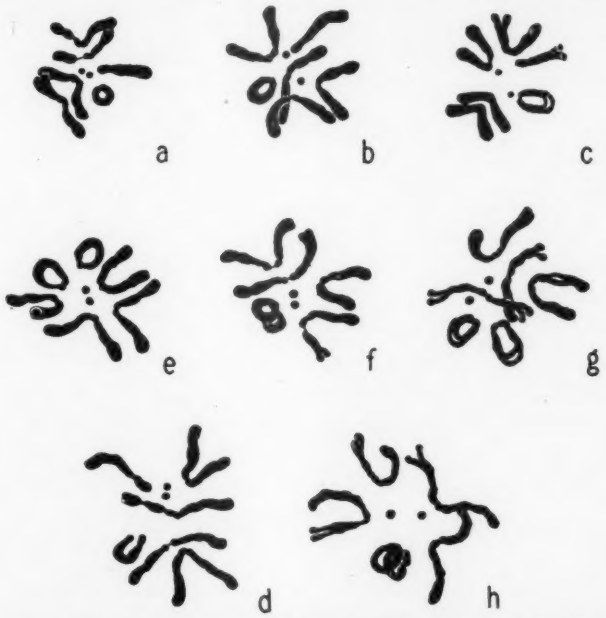

FIGURE 1.—The drawings represent metaphase-plates from the nerve cells of the ganglia of larvae of females carrying closed X^0 chromosomes. Figures a–d (from heterozygous females) contain one closed X^0 chromosome. Figures e–h (from homozygous females) contain two closed X^0 chromosomes. The drawings were made with the aid of camera lucida, at the level of the work table. The magnification is about 5300.

often appears to be closed, but at some optical levels it is somtimes slightly open on the side toward the centre of the plate, which led to its description "an almost or entirely closed and somewhat rounded letter U" (L. V. MORGAN 1926). Dr. DOBZHANSKY has very kindly made preparations and drawings of metaphase plates from the nerve-cells of the ganglia of female larvae (figure 1); in these the chromosomes are less contracted and

details of their structure are more clearly seen. In the larval nerve-cells it appears that the two ends of the curved chromosome are indeed in contact (as seen in all figures except one, figure 1d), and that they are directed toward the centre of the plate. It appears also that the ends of the chromosome are thinner than the middle, hence the apparent opening in the more contracted oogonial chromosomes, especially if the chromosome is inclined to the plane of the section, when at some optical levels only the thicker sides of the chromosome are seen.

When the chromosome lies flat in the plane of the section it appears like a ring sometimes elongated in the axis which passes between the ends. It can be interpreted as an X chromosome of which the spindle fibre end is as usual in a position near the centre of the metaphase plate and in which the usually free end of the chromosome has been brought into contact with the spindle fibre end. Thus the cytology presents an X chromosome whose ends are united to each other forming a ring or oval such that interchange between two chromatids can form a single chromatid only when exchange has taken place at two points simultaneously.

Dr. DOBZHANSKY'S figures show further that, when the chromosome divides, the split seems not to begin at the end, as occurs when the end is free, but in the middle of the closed chromosome (figure 1c and 1g).

The genetic and cytological results can be explained on the hypothesis that the unique X chromosome is a closed chromosome in which the left end is attached to its own right end near the spindle fibre attachment.

ATTACHMENT OF THE ENDS OF THE CLOSED CHROMOSOME

There is evidence from genetic tests that the point at which the ends are united is close to genes at the extreme left end and at the right end of the chromosome.

First, there is abundant evidence that the left end of the closed chromosome which carries the gene for yellow (*y*) is never separated by crossing over from the right end carrying the normal allelomorph for bobbed ($+^{bb}$), a locus near the right end. More than 11,750 female offspring of females heterozygous for *y*-closed and for *bb*, mated to *bb* males, have been examined and none showed recombination of the extreme end characters. A stock, kept by mass cultures, of females heterozygous for *y*-closed and for bobbed mated to bobbed males was made from material from which the closed chromosome had always been selected by selection of females carrying the gene for yellow. For three years no recombination of the loci of *y* and $+^{bb}$ occurred. Other stocks not marked for bobbed show linkage of the characteristics of the closed chromosome to yellow, for by selection of yellow, the closed chromosome has been kept since it was first found in 1922.

In order to test the closeness of the point of union to the gene for yellow, flies were made heterozygous for yellow carried by the closed chromosome and for broad (locus 0.6), or for prune (locus 1.0) or for allelomorphs of white (locus 1.5). No crossing over occurred between yellow and the locus for broad (among 6568 flies) or between yellow and the locus for prune (among 2138 flies) but 87 flies have been obtained that were crossovers between the loci of yellow and white. Of these, 25 were bred and all of them showed that the closed characteristics remained with yellow. Therefore, the factor or condition responsible for the chromosome being closed is so far to the left in the chromosome that 100 percent of 25 random samples of crossovers between 0 and 1.5 showed the locus of the factor to be to the left of the break.

Similarly the linkage of the point of union to the locus for bobbed was tested; 113 flies were obtained that were crossovers between carnation (4.5 units to the left of bobbed) and bobbed (near the end of the chromosome). Of many that were mated, 15 bred and showed that the characteristics of the closed chromosome accompanied the not-bobbed gene of the closed chromosome in each case, that is, the chromosome is closed by the attachment of the distal end to a point near the right end of the chromosome.

The genetic results showing the union of the ends of the chromosome to each other (locus of yellow to locus of bobbed) and the linkage of the point of union to the left end and to the right end of the chromosome are evidence for the hypothesis that the ends are physically attached and that the attachment is the condition whose effects are the characteristics peculiar to the closed chromosome.

It has been stated that a stock heterozygous for *y*-closed and for *bb* was maintained for three years by selecting females heterozygous for yellow, all of which were also not-bobbed. The chromosome carrying bobbed was also marked by scute and no scute not-bobbed female had ever been observed. At the end of that time there appeared scute not-bobbed females and also the percentage of yellow males increased, a class which had been extremely small up to that time, owing partly to inviability when the X chromosome is closed. The chromosome carrying yellow was tested and now gave among 1721 flies close to the standard percentages of both single and double crossovers that are regularly found between normal Xs, and did not produce the high percentage of patroclinous males which is found when a closed chromosome is involved. The chromosome was no longer distinguishable from a normal X.

Since the closed X^0 was derived from attached XX, the reopening of the closed X^0 suggests that it might have originated by the separation of a single X from attached XX, but this is not a satisfactory explanation, for,

if opening of the closed X^0 could occur in that way, it would have taken place frequently instead of only once.

Flies carrying a closed X^0 chromosome are poorly viable, and homozygous females and males show low fertility and sometimes abnormalities of the eyes or legs. In the reopened X^0 these peculiarities have disappeared, so they may be due to irregularities caused by the closed condition. Jagged wings often found in the presence of duplication of sections of the X chromosome have not been observed.

It has been suggested that the closed X^0 may have originated as a translocation of the yellow end of one attached X to a point near the spindle fibre attachment of the other X, or as a complete inversion in one attached X followed by single crossing over with the other. But both hypotheses imply the presence of a slight duplication at the spindle fibre end and leave open the possibility of release of the yellow end as when a detached X is derived from attached XX.

DOUBLE CROSSOVERS

The crossovers which are found among the offspring of a female carrying one or two closed chromosomes prove, when the chromosomes are completely marked, to be exclusively doubles or quadruples. It has been shown that among at least 11,750 flies from heterozygous mothers and in various heterozygous stocks those chromosomes that retained the left end of the closed chromosome carrying yellow also retained the right end carrying not-bobbed, and this linkage can be assumed to hold for flies that cannot be rated for bobbed. (In the first count of experiment A_1 two flies are recorded as recombinations of yellow and bobbed. Subsequently, whenever there was doubt as to the classification of bobbed, the fly was tested and among those that bred none showed recombination. The two exceptions had almost certainly been wrongly classified.) Using this assumption in classifying it was found that among 16,914 flies (table 10), all the crossovers, of which there were 2,211, were doubles or quadruples and none were singles or triples.

The genetic results show that if interchange takes place between a closed X^0 chromatid and a normal X chromatid simultaneously at two points, viable zygotes of both complementary classes may reach the adult stage.

FREQUENCY OF DOUBLE CROSSOVERS

The attachment of the ends of an X chromosome affords a new situation for crossing over since single crossovers are not represented by the usual classes. It is of interest to know whether there is an effect on the frequency of double crossing over for the whole chromosome and for different regions of the chromosome. Frequencies of double recombinations have been determined by the method of alternated backcross devised by

BRIDGES and OLBRYCHT (1926) in mapping the X chromosome. Their results will be referred to as the alternated X-ple or a.X. values. The chromosomes used by them were so marked that crossing over could be followed in six regions, from scute at 0 to forked at 62 with little or no chance of double crossing over between the marked loci. The character bobbed near the extreme right end of the chromosome was not included but when the experiments with the closed chromosome were made, bobbed was introduced, thereby bringing under observation a seventh region of the chromosome to the right of forked.

Material descended from the a.X. stocks of BRIDGES and OLBRYCHT was used to make stocks heterozygous for X^0 *y cv v f* and for X^0 *y ec* ct^6 g^2 and also to make the apposers and the testers that were used. The symbol X^0 is used to designate the closed chromosome.

The combined experiments with marked flies heterozygous for closed X^0 involved 16,914 flies not including those of non-disjunctional classes. As already stated, yellow in the closed chromosome is always linked to not-bobbed so that among males in which bobbed does not show, because of the normal allelomorph of bobbed carried in the Y chromosome, and among flies of experiment B_2, where the tester was not bobbed, all yellow chromosomes were rated as not-bobbed, and not-yellow as bobbed. The apparent single crossovers were in this way found to be doubles that involved the seventh region.

It is apparent from the counts of observed offspring of heterozygotes (appendix, table 10) that factors for differential viability are present. Flies containing a closed X^0 (yellow males and females heterozygous for yellow) occur less frequently than the complementary types, class for class, and furthermore the difference is proportionately greater among crossovers than among non-crossovers. The ratio of the frequency of non-crossovers of X^0 classes to non-crossovers of X classes in the combined experiments is 73 to 100, and for crossovers the ratio is 29 to 100 in bottles containing a single female parent.

Viability probably varies with the conditions of the environment. When only the eggs laid by one heterozygous female in one day developed in a bottle, the ratio of X^0 to X non-crossovers in one experiment was 89 to 100. In mass cultures of heterozygous females back-crossed to normal X males, there are few if any yellow males whereas with uniform viability they are expected to equal nearly 1/4 of the population. Homozygous cultures hardly survive even under favorable conditions.

The mortality occurs partly at least in the pupal stage. Of 62 dead pupae found in pair cultures of heterozygous females, 87 percent belonged to the classes carrying a closed X^0; they were either X^0 males or females heterozygous for X^0.

In the first two experiments (A_1 and A_2) *y cv v f* females heterozygous for X^0y and *sc* were mated to *ec* ct^6 g^2 *bb* males, with or without scute, and F_1 females were mated singly to X_8 males which carried all of the recessive genes except scute. Cultures that gave yellow males in F_2 are included in table 10 and those that gave not-yellow (or scute) sons were used as controls (appendix, table 11). The control involved a total of only 2,623 flies, but recombination percents (table 2) corresponded well enough with the a.X. values to show that the stock had not changed since it had been used by BRIDGES and OLBRYCHT, and more significant results of the larger a.X. experiment could be used. The map distances derived from the a.X. values are not the same as the present standard values for the X chromosome which have been more recently obtained from a wide range of material, but since the stocks used in the experiments were made from alternated X-ple material, the a.X. values are suitable for comparison.

TABLE 2

Recombination values of the eight chief characters used in the experiments with the closed chromosome obtained from two control experiments and from "a.X." data of BRIDGES *and* OLBRYCHT.

	REGION							TOTAL NUMBER OF FLIES
	sc-ec 1	*ec-cv* 2	*cv-ct^6* 3	*ct^6-v* 4	*v-g* 5	*g-f* 6	*f-bb* 7	
From a.X.	6.8	9.6	8.2	14.8	11	11.4		20,786
From two controls	7.2	10.6	8.3	16.2	11.1	12.7	10.5	2,623 585

In order to compare the rates of crossing over in different regions of the chromosome the total percent of recombination observed in each region (appendix, tables 10 and 12) has been divided by the number of the a.X. map units that represent the length of the region. The horizontal axis of diagram 1 represents the chromosome map, the vertical axis the frequency of crossing over expressed as percent per map unit of the total number of observed recombinations for each region. In order to eliminate the factors for differential viability among offspring of heterozygotes, the percents of recombination have been calculated separately for offspring of the two classes, with and without a closed X^0.

The frequency of crossing over in the first and seventh regions of flies *homozygous* for closed X^0 cannot be observed directly because no new characters can be introduced at the ends of the chromosome; double crossovers involving either of the two end regions therefore appeared to be singles and the doubles involving both end regions appeared to be noncrossovers. The percent of apparent singles from females homozygous for closed X^0 is 3.42. The percent of crossovers involving either the first or the seventh region among flies that received the closed X^0 from *hetero-*

zygous mothers is 3.44, a close agreement for both regions together. In default of direct values for doubles involving these regions, the singles have been allocated as doubles in the same proportions as were found in experiments A_2 and Aa, in which the closed X^0 came from heterozygotes. Similarly a certain percentage of phenotypical non-crossovers from homozygotes, corresponding to the percentage of crossovers from heterozygotes involving both the first and seventh regions, have been rated as crossovers of the 1, 7 class. The percentages obtained are nearly equal to the percentages that closed X^0 crossovers are of the closed X^0 class from heterozygotes.

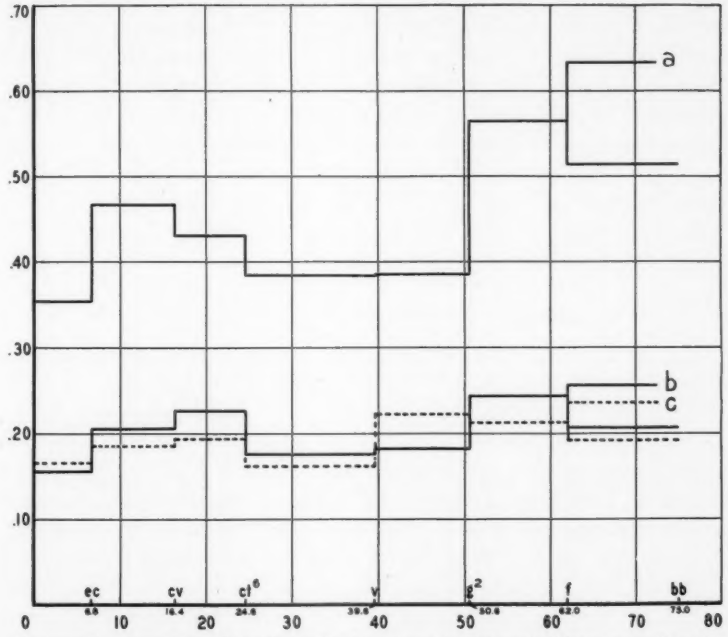

DIAGRAM 1.—Frequency of double crossing over found in flies marked for seven regions of the X chromosome. (a) Percents per map unit of double crossovers from females heterozygous for closed X^0 as shown by offspring not carrying a closed X^0. (b) Same from offspring carrying a closed X^0. (c) Percent per map unit among offspring of females homozygous for closed X^0.

The ratio of the percent of crossing over among flies of the closed X^0 class from heterozygotes to the percent of crossing over among the normal X class is a little less than 1:2 for every region of the chromosome. It will be shown later that this ratio is due in all probability to a lethal class among closed X^0 crossovers, and in the comparisons with normal crossing over that will follow, values for the normal X class will be used.

Crossing over in heterozygous and in homozygous X^0 females (diagram 1) is lowest in the fourth region and increases on both sides; after a maximum in region 3 or 2, there is a decrease to the left. A low rate of crossing over at the left end of the X^0 chromosome has been found, as already stated, in experiments with chromosomes marked close to yellow, for no recombination occurred of yellow and broad or yellow and prune.

It is difficult to determine the percent of crossing over per unit of map distance at the right end because the distance from forked to the end of the chromosome was not known for the a.X. chromosome, and that distance has been found from unrelated experiments to be very variable. If the value of about 13 units found by STERN (1926) is used, the percent per unit drops for the seventh region, but, if 10.55 units found in the small control experiment is used, the curves rise. In experiment B_1 and B_2 when a new dominant character Beadex-2, 3.3 units to the right of forked, was introduced into the apposing chromosome, the drop found for 13 units from forked to bobbed was progressive from forked to beadex to bobbed. The most recent values for forked to beadex and beadex to bobbed obtained from many different stocks are 2.6 and 6.6 respectively. Using these values the percent of crossing over per unit increases progressively from the fourth region to the right end of the chromosome.

COMPARISON WITH A NORMAL X CHROMOSOME

In order to make percentages derived from alternated X-ple values comparable with those from experiments with closed X^0, some corrections are necessary. Assuming that single crossovers with the closed chromosome are inviable, only double crossover frequencies for two normal Xs can be compared with the crossover frequencies in females heterozygous for normal X and closed X^0; accordingly, percentages of doubles from a.X. have been computed as of the total number of flies minus the singles and triples, except that the undetectable singles of the seventh region are not subtracted, which makes the percentages for a.X. too low. Furthermore, since in the a.X. experiments the seventh region was not marked, crossovers that were in fact doubles involving the seventh region appeared to be singles and have been subtracted as such from the grand total when singles have been subtracted. It is therefore necessary for comparison to subtract from the closed X^0 totals all the doubles involving the seventh region. Exceptional classes have also been subtracted.

The results obtained from the corrected data are given in table 3. They show that the frequency of total double crossing over in heterozygotes, as indicated by the normal chromosomes that are recovered, is less than the frequency of double crossing over between normal Xs.

A more detailed comparison has been made for different regions of the chromosome. The curves of diagram 2 have been obtained from the corrected data showing the percents of total crossing over per map unit for six regions based on doubles and quadruples when the seventh region of the chromosome is not under observation; the percents for a.X. being as explained a little too low. Disregarding for the present the left end of

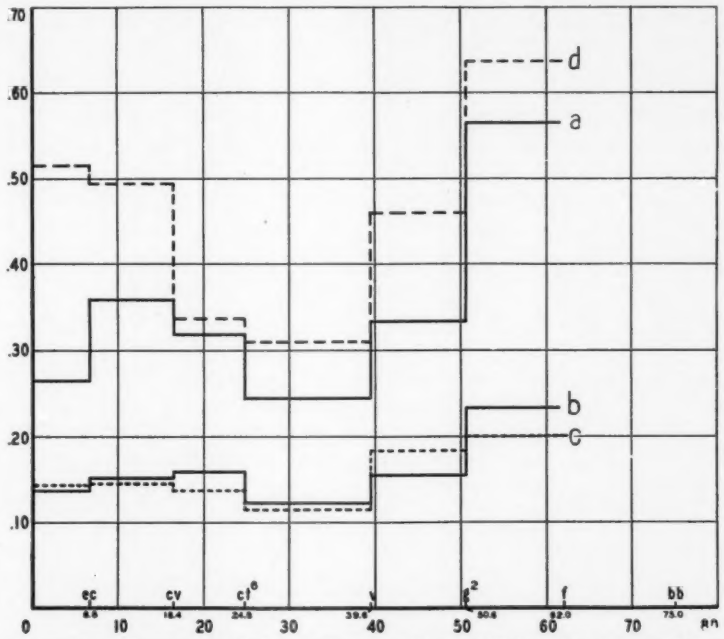

DIAGRAM 2.—Frequency of double crossing over with closed X^0 from data corrected for comparison with alternated X-ple (a.X., BRIDGES and OLBRYCHT) in six regions of the X chromosome (a, b, c as in diagram 2, d percents per map unit of double crossovers from corrected data of a.X.)

the chromosome it is apparent that the percent of crossing over in the normal class from heterozygotes is for every region to the right of *cv* somewhat, though not constantly, lower than the percent of crossing over between normal Xs. From *cv* to *ct* the percents are most nearly equal, and the relative percents for heterozygotes fall progressively from *ct* to *g*. The percents for homozygous closed X^0 from *cv* to *g* follow more closely the changes in percents for normal Xs, maintaining a nearly 1:3 ratio; this continues in the *g* to *f* region, in which the percent from heterozygotes again approaches more nearly the normal X value. The result may be stated in another way; the ratios of the frequencies of double crossovers

from two normal chromosomes and from a closed and a normal chromosome vary in different parts of the chromosome from *cv* to *f*, while the relative frequencies of double crossovers from chromosomes that are alike, either both normal or both closed, are more constant from *cv* to *f*.

TABLE 3

Relative percents of crossovers from females heterozygous for closed X⁰ and from females homozygous for normal X or for closed X⁰.

	CORRECTED TOTAL ADULT FLIES								
	REGION NOT OBSERVED								
	SEVENTH	FIRST	PERCENT OF DOUBLE CROSSOVERS OF SIX REGIONS AS OF CORRECTED TOTAL	RATIO OF PERCENT OF DOUBLE CROSSOVERS FROM X⁰/X TO PERCENT FROM X/X	TOTAL OBSERVED REGULAR ADULT CLASSES	PERCENT OF DOUBLE CROSSOVERS OF SEVEN REGIONS AS OF TOTAL OBSERVED REGULAR ADULT CLASSES	TOTAL POPULATION	PERCENT OF SINGLE CROSSOVERS AS OF TOTAL POPULATION	RATIO OF PERCENT OF SINGLE CROSSOVER CHROMATIDS FROM X⁰/X TO PERCENT FROM X/X
a.X. $\frac{X}{X}$	11111*		14.0*				20786	46.7	
X⁰/X X	9488		10.8	0.77					
X⁰	6564		4.95	0.35					
Homozygous X⁰	2580		4.76						
Control $\frac{X}{X}$		1420*	20.7*						
X⁰/X X		9924	14.72	0.71	10168	16.7			
X⁰		6674	6.54	0.32	6746	7.54			
Homozygous X⁰		2616	6.46		2646	7.1			
Exp. J_2 X⁰/X X					1928	20.4	*3135*⁺	*30.8*⁺	0.66
X⁰					1438	9.4			

* Corrected totals for a.X. and control are too high because they include undetectable singles of the seventh and first regions respectively; the percents are correspondingly too low (see text).

⁺ Computed for first 6 days of laying (see table 7).

For the seventh region the percents of crossing over for the closed chromosome were directly compared with those from the small control experiment, which had been made to check the material for a.X. values (see table 3). In the control, the first region was not marked and corrections were made similar to those made for comparison with the a.X chromosome, unmarked in the seventh region. Again the percents from the control are a little too low owing to undetectable singles of the first region being included in the total. This does not affect the conclusion that there is no relative decrease in crossing over at the right end of the closed X⁰ (table 4). Ratios for the normal X class from heterozygotes show a decrease for the *v-g* region consistent with that found in the comparison with a.X. (diagram 2) and an increase toward the right end. The results for closed X⁰ classes are not entirely consistent.

TABLE 4

Ratios of the percents of crossing over in regions 2 to 7 in flies heterozygous and homozygous for closed X⁰ to the percents of crossing over obtained from a small control experiment. The percents are obtained from corrected data and are based on the frequency of observed double and quadruple crossovers.

		y-ec	*ec-cv*	*cv-ct*	*ct-v*	*v-g*	*g-f*	*f-bb*
X/X⁰	X		0.60	0.72	0.72	0.66	0.71	0.80
	X⁰		0.27	0.38	0.31	0.38	0.29	0.34
Homozygous	X⁰		0.23	0.32	0.28	0.32	0.26	0.21

A reduction in crossing over at the left end of the chromosome beginning in the second or first region was found to occur with a closed X⁰ when all regions were included (diagram 1), and appears also when the crossovers involving the seventh region are not included (diagram 2). Crossing over between two normal Xs on the other hand increases progressively from the region that is the minimum for all the chromosomes, continuously, to the extreme left end of the chromosome.

The attachment of the left end of the closed chromosome to the right end is in a sense a translocation, and, as in translocations generally, there is a decrease in crossing over near the region of attachment; but this is not a reduction due to the conflict of attractions of genes of the sort described by DOBZHANSKY (1931) in the true translocations. The X chromosomes are intact and neither chromosome is attracted to parts of another that are controlled by two different spindle fibres as in cases cited by him. The results are in accord with the view of BEADLE (1932) and of OFFERMAN and MULLER (1932) (see also GRAUBARD 1931) that proximity to the spindle fibre attachment is a factor that reduces crossing over, for in the closed chromosome the attachment of the yellow to the bobbed locus has brought the left end of the chromosome near to the spindle fibre attachment.

INVIABLE CROSSOVERS

Crossing over, or exchange of segments between chromatids, takes place in Drosophila in the four-strand stage (BRIDGES 1916, ANDERSON 1925, L. V. MORGAN 1925, STURTEVANT 1931). In females heterozygous for closed X⁰ and normal X two chromatids have no free end which imposes a new condition on the recovery of crossovers. For, if a closed and a normal chromatid exchanged segments at one level only (diagram 3, S), the result would be two tandem chromatids, attached together, because the "yellow" or distal end of the original closed chromatid is inseparable from its spindle fibre end; the chromatid complex would have a spindle fibre attachment at one end (derived from the normal X) and a second attachment (derived from the originally closed X⁰) in the middle of the new double chromatid.

If the double complex passes entirely into one nucleus, non-disjunctional females with united Xs would be expected. Non-disjunctional females are not unusually frequent and a few that were tested contained separate X chromosomes, not a double complex. It is therefore assumed that a complex carrying two spindle fibre attachments may be lethal and some evidence for the assumption will be given later. DOBZHANSKY (1931) has pointed out that among translocations some would be expected leading to the formation of chromosomes having more than one spindle fibre attachment and there are no descriptions of such chromosomes in the literature.

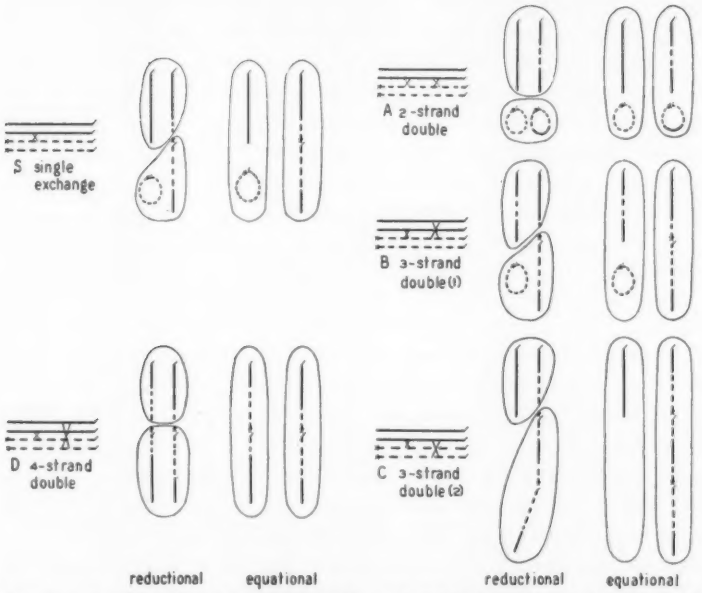

DIAGRAM 3.—Analysis of chromatid formation and distribution at the first meiotic division, when exchanges between homologues have taken place.

Double exchange (that is, two exchanges at the same time, not at the same level [ANDERSON, 1929]) may involve two or three or all four of the chromatids at once. Diagram 3 represents the four possible second exchanges between homologous strands that may occur simultaneously with a first exchange between homologues. A 2-strand double exchange leaves all the chromatids separate from one another and may result in a non-crossover and a double crossover of each kind, closed and normal (diagram 3A). The term normal designates a chromatid which contains the spindle fibre end derived from a normal chromosome.

A 4-strand double exchange is equivalent to two simultaneous single exchanges (diagram 3D) and is by hypothesis therefore lethal for all four chromatids.

A 3-strand double exchange may be one of two kinds. If two of the three strands are normal chromatids (each with a free end, diagram 3B) there may be recovered a closed X^0 non-crossover and a normal X double crossover since the exchanges lead from one normal spindle fibre end to the free end of the other normal chromatid. From the other normal spindle fibre end, however, exchange leads to the end of the closed chromatid attached to its own spindle fibre end, and the result is a lethal double complex.

If two of the three exchanging strands are closed chromatids one non-crossover normal chromatid may be recovered, but the other three chromatids will all be united (diagram 3C) and will *a fortiori* be lethal.

Closed X^0 double crossovers can therefore be derived from 2-strand double exchange only, but normal double crossovers are derived from both 2-strand and 3-strand exchange. It has been noted that the ratio of the percent of double crossovers among closed X^0 F_1s to the percent of double crossovers among normal X F_1s is very nearly the 1:2 ratio to be expected if exchange between homologous strands is at random.

The 1:2 ratio for closed X^0 to normal X double crossovers shows that homologous strands cross at random when two or three strands are involved in double exchange. The closed X^0 tells nothing about the frequency of 4-strand double exchange but EMERSON and BEADLE (in press) have found from data for attached X that 2-strand and 4-strand double exchanges are equally frequent. This result and the equality between 2-strand and 3-strand exchanges shown by closed X^0 are to be expected if when there is crossing over at one level a second crossing over at another level takes place by random exchange of homologous chromatids. The expectation is the same if triple exchange is random (see diagram 4 and table 5). It may be concluded therefore that exchange between homologous chromatids is at random.

PATROCLINOUS MALES

Patroclinous males occur in the proportion of 1 to 3 normal X double crossovers. This is chosen as a critical ratio because of the good viability of the normal class and because almost all double crossovers are derived from double exchange which perhaps is the source also of almost all patroclinous males. It has been found that closed X^0 double crossovers are half as frequent as normal X doubles and it has been shown that half of the closed X^0 doubles expected by random exchange between homologous chromatids are probably inviable. An analysis of the distribution of chromatids shows that patroclinous males may arise at the expense of

closed X^0 doubles. A closed X^0 double crossover from 3-strand double exchange involving two closed X^0 chromatids (diagram 3C) would be inviable because it would be united with two other chromatids, in a lethal triple complex. If at the first meiotic division two of the chromatids that are going together to one pole (either equationally or reductionally) drag the third chromatid away from the other pole, one daughter nucleus might

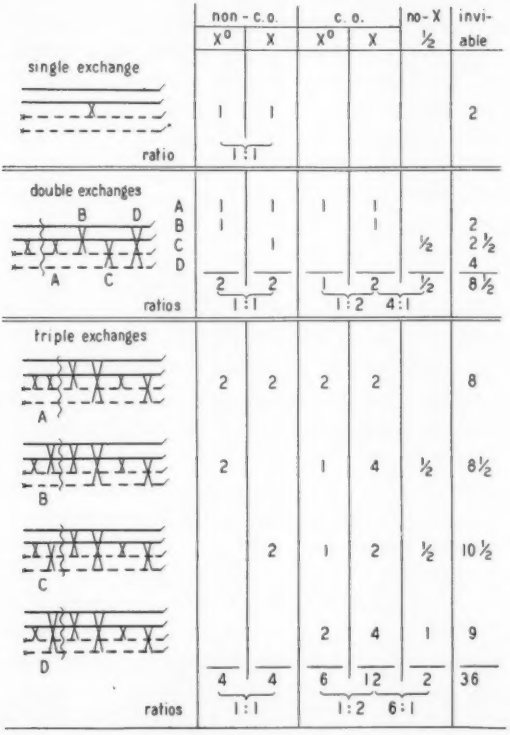

DIAGRAM 4.—Chromatids and ratios to be expected from random exchanges between homologous chromatids in heterozygotes for closed X^0 and normal X, on the assumptions that double and triple complexes are lethal and that patroclinous males are mainly derived from double exchange C. A closed chromatid is represented by a broken line and the end marked "x" is attached near the spindle fibre to the same chromatid. On the right of the wavy lines are represented the different exchanges that might occur simultaneously with the exchange or exchanges on the left of the line, by double and triple exchange.

contain only one X chromatid and at the second division one no X nucleus might be formed. If such a nucleus remained in the egg, it would, if fertilized by Y sperm, be lethal in the egg stage according to LI (1927), and if fertilized by X sperm it would contain the chromosome complement

of a patroclinous male. If patroclinous males have this origin the expected frequency from random double exchange is 1 to 4 normal X doubles, which is lower than the observed frequency of 1 to 3. (From infrequent triple exchange the expectation, if patroclinous males are due to formation of triple complexes, is still lower; see diagram 4 and table 5).

TABLE 5

Classes and Ratios; (1) *To be expected* (*a*) *If homologous strands of closed* X^0 *and normal X cross at random and if patroclinous males are derived from one kind of 3-strand double exchange, and united chromatids produce inviable eggs.*

TYPE OF EXCHANGE	NON-CROSSOVERS X^0 : X	DOUBLE CROSSOVERS X^0 : X	NO X EGGS BY X SPERM (PATROCLINOUS MALES)	INVIABLE EGGS	RATIO OF X NON-CROSSOVERS TO INVIABLE EGGS
none	1 1	0 0		0	
ratio	1:1				1:0
single	1 1	0 0		2	
ratio	1:1				1:2
double	2 2	1 2	0.5	8.5	
ratio	1:1	1:2	4:1		1:4
triple	4 4	6 12	2	36	
ratio	1:1	1:2	6:1		1:9
		(*b*) *If all strands cross at random*			
none	1 1			0	
ratio	1:1				1:0
single	6 8	0 0		10	
ratio	75:100				0.8:1
double	10 16	1 2	2.5	28.5	
ratio	62:100	1:2	4:5		1:2
triple	19 39	19 29	11	139	
ratio	50:100	2:3	3:1		1:3
		(2) *Observed*			
ratio	1:1	1:2	3:1		1:1.2

The source of some of the patroclinous males may therefore be looked for in other kinds of exchanges and although little is known about the separation of chromatids at meiosis in Drosophila a formal anslysis can be made, presenting at least some of the problems involved.

Two-strand double exchange (diagram 3A) results in four single chromatids which may be distributed regularly among the four maturation nuclei. Four-strand double exchange (diagram 3D) results in two double

complexes. The remaining two kinds of exchange, namely single exchange and 3-strand double exchange involving two normal X chromatids (diagram 3S and B) are alike in that each results in a single normal chromatid (in the second case a double crossover), a single closed chromatid, and a double complex with two spindle fibre attachments. The non-crossover

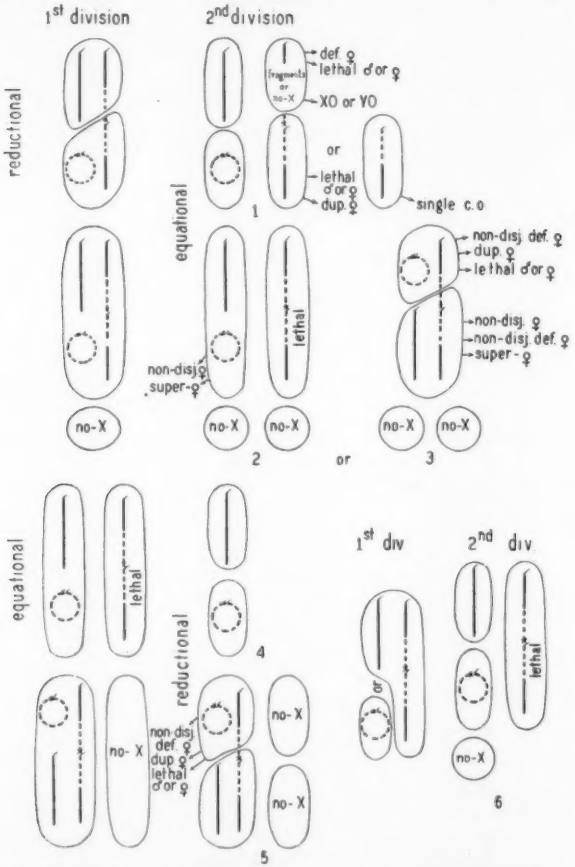

DIAGRAM 5.—Schemes of meiotic divisions in heterozygotes after single exchange (S of diagram 3) or 3-strand double exchange that involves two normal X chromatids (B of diagram 3).

chromatids from single exchange (S) should be recovered to account for the frequency of observed non-crossovers and the 1:1 ratio between the normal X class and the closed X^0 class. The normal X double crossover from exchange B should be recovered to account for the 1:2 ratio of closed X^0 doubles to normal X doubles.

If the reductional division takes place first, each daughter nucleus will contain one of the single chromatids resulting from exchanges S or B, and if these single chromatids are to be recovered the nuclei must be viable. The first division can be truly reductional (that is, the sister chromatids can remain together at the fibre end) only in case the normal component of the double complex is torn apart, and the complex is not lethal in the sense that has been assumed (see diagram 3 and 5). If chromatids are severed in this way, one of the daughter nuclei at the second (equational) division might receive the normal X chromatid and the other might receive a fragment or fragments of a chromatid; a nucleus with a fragment and its spindle fibre attachment might produce an inviable zygote or, if fertilized by X sperm, might produce a female with a deficiency in one of her X chromosomes. No such flies have been observed. If a fragment with a spindle fibre attachment were small, and sometimes went with the whole chromatid at the second division, duplications would be expected among the offspring, but these were not found. If all fragments were lost a nucleus containing no X chromatid might contribute to the class of patroclinous males.

The other daughter nucleus of the first reductional division would contain the spindle fibre ends of the closed chromatids (diagram 3S); one chromatid would be a closed non-crossover and the other chromatid might carry an extra piece torn from the normal chromatid that had become united to it by crossing over. The second division would give a nucleus with a closed chromatid, and one of a kind that would produce inviable zygotes or females with duplications, which are not found. If the united chromatids separated at the first division at their point of attachment single crossovers with the fibre attachment of the original closed X^0 would be expected but are not found (diagram 5, scheme 1). Similarly duplications and single crossovers might sometimes be expected from 4-strand double exchange (diagram 3D).

Three strand exchange that involves two closed X^0 chromatids (diagram 3C) and that results in a normal X chromatid and three chromatids united together might be expected to produce similar exceptional classes by rupture of chromatids if the first division is reductional. Or the three united chromatids might all go into one nucleus, giving the results already formulated.

If however the first meiotic division were equational, the recovery of single chromatids after exchanges S and B would involve no united chromatids whenever the double complex segregated from the two single chromatids (diagram 5, scheme 4). Assuming that double complexes are lethal no viable chromatids would be expected after 4-strand double exchange with this kind of equational division.

But on chance association of spindle fibre ends, the ends of a free and of an attached chromatid would go together at a first equational division as frequently as the ends of the free chromatids would separate from the ends of the attached chromatids. The resulting nuclei after the second division would be of the same kinds as after a first reductional division (see diagram 5, scheme 1) and classes would be expected that have not been observed.

If all four X chromatids went into one first daughter nucleus and no X into the other, the first division would be neither equational nor reductional. Following out such a possibility to the second division the no X nucleus might divide into two no X nuclei and the other nucleus divide also. But whether such a nucleus underwent the second division reductionally (diagram 5, scheme 5) or in either of two ways equationally (diagram 5, scheme 2 or 3) non-disjunctional and other classes that have not been observed should result.

It appears that the kind of assortment of chromatids that best fits the observed classes, except for the excess of patroclinous males, is one which segregates the free chromatids from a double complex at the first division (diagram 5, scheme 4).

If exceptionally, at the first division, two united chromatids and only one of the single chromatids went together into one nucleus and the single chromatid separated from the double complex at the second division (diagram 5, scheme 6), the 1:1 ratio between non-crossovers and the 1:2 ratio between double crossovers would be maintained and there would be an increase in the frequency of no X eggs, and therefore of patroclinous males, derived from one half of the nuclei that after the second division contained no X.

The observed percent of double crossovers from homozygous closed X^0 females is in agreement with expectation on the assumptions that have been made from a study of heterozygotes. Two-strand exchange would give viable double crossovers as in heterozygotes and 3-strand exchanges would produce patroclinous males. The double crossovers recovered should therefore be the same percent of the total regular classes as are closed X^0 doubles of the total closed X^0 class from heterozygotes which was realized (table 3 and diagrams 1 and 2). The percent of patroclinous males from homozygotes, if they are derived mostly from 3-strand exchange, would be expected to equal the percent of patroclinous males as of a total consisting of the closed X^0 class plus the patroclinous males from heterozygotes. Homozygotes gave 12 percent among a total of 2121 flies (table 12). Heterozygotes of experiment Aa gave 10 percent among 2087 flies of the corresponding classes and 9 percent occurred among another total of 2588. This is a close agreement but viability of the closed

X^0 class as compared with that of the normal X class was exceptionally low in both the experiments with heterozygotes. In another experiment (B_2, table 10) in which viability of closed X^0 flies as compared with viability of normal X flies was almost twice as good, patroclinous males were only 5.6 percent of a corresponding total of 1534. There was no measured control of the viability of F_1s from homozygotes but among 3 cultures there was an average of only 62 flies of regular classes from a culture; larvae were numerous, but not pupae and many of the pupae were dead; also many flies that hatched did not survive. It may be concluded that, although differential viability obscures the result, the unexpectedly high percentage (12 percent) of patroclinous males from homozygotes may not be at variance with the assumption that they originate mainly from eggs in which triple chromatid complexes are formed by crossing over.

INVIABLE EGGS

In Drosophila, the first meiotic division is not completed until after the sperm has entered the egg (HUETTNER 1924, GUYÉNOT and NAVILLE 1929) and since crossing over takes place at the four strand stage, which occurs at meiosis, irregularities due to crossing over should affect the egg stage (see PLOUGH 1921, GOWEN 1929a and b). It follows that a certain proportion of the eggs laid by flies heterozygous for closed X^0 and normal X would be expected to be inviable if double or triple crossover complexes are lethal as has been assumed, but that if crossing over were reduced, or prevented, a corresponding reduction in inviable eggs would be expected.

Dr. JACK SCHULTZ suggested a comparison of the viability of eggs from females heterozygous for closed X^0 and normal X with viability of eggs from females in which the normal X has been replaced by an inverted X called Δ-49 (MULLER and STONE 1930, OFFERMAN and MULLER 1932) preventing nearly all crossing over.

The flies used were X^0 y/g^2 females mated to y g^2 bb males, X^0 $y/$Δ-49 $cm \pm bb$ females mated to y Δ-49 cm bb males, and for control Δ-49/g^2 females by g^2 males. A single female was put into a vial with many males, and the next day transferred to a bottle in which was placed food darkened by lampblack and contained in the depressions of handles of two paper spoons. On the first day following and on five successive days the spoons were removed and new spoons put into the bottle with the flies; the eggs on the spoons that had remained a day with the flies were counted and the spoons put into new bottles supplied in the regular way with fly food. On the fourth day eggs still unhatched were counted. Eight females of each kind were mated and the eggs that were laid on the first six days were followed in this way.

The result is consistent with the hypothesis for the percent of inviable eggs was reduced from 31.3 percent to 6.3 percent when crossing over with

a closed X^0 was reduced by the inversion in the Δ-49 chromosome (see table 6).

TABLE 6

Percent of classes representing crossovers from females carrying inverted and closed X^0 chromosomes (deduced values in italics).

EXPERIMENT	HETEROZYGOTE	PATROCLINOUS ♂♂	SINGLE CROSSOVER	DOUBLE CROSSOVER	INVIABLE EGGS	TOTAL
A.H.S. (unp.)	ClB/X	0.5				1743
	ClB/X		0.5			
C	ClB/X^0	0.52		0.1		6122
L_1	Δ-49/X	0.27				1798
L_2	Δ-49/X^0	0.33				4150
K_1	Δ-49/X^0	0.5				2099
M	Δ-49/X	0.3				1329
J.S. (unp.)	Δ-49/X		13.0	0.4		1309
J_3	Δ-49/X				3.2	3498
J_1	Δ-49/X^0			0.0	6.3	3383
J_2	X^0/X		0.0		31.3	3326
		2.36		*14.3*	*33.2*	*3135*

Furthermore the presence of some inviable eggs from heterozygotes for closed X^0 and Δ-49 is consistent with the conclusion reached from analysis of maturation divisions that usually two single chromatids segregate from a double lethal complex. Dr. SCHULTZ found that heterozygotes for Δ–49 and normal X gave 13 percent of single crossovers due to single exchange in the region not included in the inversion of the Δ-49 chromosome, and only 0.4 percent of doubles (which involved the left end of the chromosome). The kind of segregation cited has been shown to be the only scheme on which inviable eggs would regularly be produced by single exchanges (see diagram 5). Since Δ-49 gave 3.2 percent of inviable eggs (due to unknown causes) in the control with normal X (experiment J_3, table 6), it may seem that 6.3 percent of inviable eggs from heterozygotes for Δ-49 and closed X^0 is not high enough to correspond with 13 percent of single crossovers from heterozygotes for Δ-49 and normal X; that they do correspond is however the most consistent conclusion especially since single crossover chromatids from closed X^0 and normal X seem to be only 0.66 times as frequent as single crossovers from normal Xs.

A second conclusion from analysis of meiotic divisions, namely, that patriclinous males mostly result from double exchange, is further shown by the percents of patroclinous males found from flies heterozygous for closed X^0 and for Δ-49 or ClB, another longer inversion.

The frequency of non-disjunctional classes, especially of males is known to be high among offspring of flies heterozygous for inversion and normal X (see table 6). For example in experiment L, Δ-49/X females gave 0.27

percent of patroclinous males, and ClB/X females gave 0.5 percent as computed from unpublished data of Dr. STURTEVANT. Flies heterozygous for closed X^0 and the inversion Δ-49 gave 0.33 percent in the controlled experiment L, and flies heterozygous for X^0 and ClB gave 0.52 percent; that is when inversions are present closed X^0 gives the percents of patroclinous males that are normal for the inversions and not the high percents that are characteristic for heterozygotes of closed X^0 and normal X. The percent of all crossovers from ClB/X females is said to be not more than 0.5 percent and ClB/X^0 females gave only 0.09 percent of double crossovers. The reduction of double crossovers by the inversion of a chromosome is accompanied by decrease of patroclinous males among offspring of flies carrying closed X^0. That patroclinous males are seldom derived from single exchange is indicated because there is no increase in their percentage from closed X^0/Δ-49 females as compared with that from normal X/Δ–49 females although some single exchange occurs with the inversion Δ–49.

The results just cited in regard to patroclinous males and inviable eggs obtained from flies heterozygous for closed X^0 and inversions, and the results from flies heterozygous for closed X^0 and normal X, namely the absence of increase in the frequency of non-disjunctional females, the absence of a class with united Xs, and of duplications and deficiencies, the near agreement between the frequency of patroclinous males and 1/4 of the frequency of normal X double crossovers, suggest that in eggs containing closed X^0 there is usually segregation of single chromatids from united chromatids at the first maturation division when two chromatids become united by single exchange. Such a segregation is equivalent to one kind of equational division.

FREQUENCIES OF ALL CLASSES

Whatever the mechanism by which exchange between chromatids produces lethal effects in flies heterozygous for closed X^0 the inviable eggs and patroclinous males together should, on the hypothesis that inviability of eggs is due to lethal effects of crossing over, correspond to single crossovers, triple crossovers, and about ¼ of the double crossovers from normal Xs. Patroclinous males were 2.36 percent of the deduced total population in the egg count experiment, and inviable eggs were 33.2 percent of that total (see table 7); the classes enumerated are therefore 35.56 percent of the total, a value which is low compared with normal X; but it is of the order to be expected, and is in agreement with other results with closed X^0 that show a lower rate of crossing over than that which occurs between normal Xs. From corrected data for alternated X-ple, the percent of doubles among the normal X offspring of closed X^0 heterozygotes is

0.77 times the percent of doubles from the a.X. control, and the percent of computed singles as of the whole population in the egg count experiment is 0.66 times the percent of singles in a.X. (see table 3).

Values from preliminary unpublished data recently obtained by Dr. BRIDGES in an experiment (425 C) with completely marked Xs are compared in table 8 with deduced values from the egg count experiment J_2.

TABLE 7

Observed and deduced frequencies of classes of experiment J_2. Offspring of X^0y/g^2 females by yg^2bb males. Deduced values are in italics.

Total no. of eggs 3326	adult flies	X	non-crossovers	849		27.1 percent
			doubles	224		7.15 percent
		X^0	non-crossovers	754 = 89 percent of 849		24.0 percent
			doubles	73	(89 percent of 112 = 99)	2.33 percent
		patroclinous males	XO assumed to be mostly from 3-strand double exchange (2)	74	(74:224 = 33:100) (56:224 = 25:100) 56 may be expected from 3-strand double exchange (2)	2.36 percent
	inviable in late stages	X^0	X^0 non-crossovers	*95*	(849–754)	3.03 percent
			X^0 2-strand crossovers	*26*	(on basis of viability of non-crossovers, 99–73)	0.83 percent
	inviable eggs	1040	inviable complexes from exchanges (single crossovers)	*966*		30.81 percent
			YO assumed to be of same origin and frequency as observed XO	*74*		2.36 percent
				3135		
	not accounted for from all classes			191		
				3326		

Non-crossovers from closed X^0 heterozygotes are 1.38 times as frequent as from normal flies. The deduced value for single crossovers in J_2 is 0.65 times the percent of singles from normal Xs. The percent of double crossovers in experiment J_2 is based on observed crossovers among flies carrying normal X; crossovers in experiment J_2 occurred with the maximum frequency that was observed among various experiments (see table 10,

appendix, and table 3); corrected for average frequency the value is 11.2. The consistently lower percentage of normal X doubles in every region of the chromosome in the more extensive experiments with heterozygotes as compared with alternated X-ple (diag. 3) seems to show that the true value for frequency of double crossovers from heterozygous X^0 is lower than for flies carrying only normal Xs.

TABLE 8

Percents of classes derived from females heterozygous for closed X^0 and from normal females in two 6-day experiments. Percents of experiment 425 C are computed from preliminary unpublished data of C. B. Bridges on crossing over of completely marked X chromosomes. Deduced percents from experiment J_2 are from table 7.

EXPERIMENT	P_1	NON-CROSSOVER	SINGLE AND TRIPLE CROSSOVERS	DOUBLE AND QUADRUPLE CROSSOVERS	TOTAL
J_2	X^0/X	*54.2*	*30.8*	*14.3* (11.2)*	*3135*
425 C	X/X	39.3	47.6	12.2	1243

* Percent deduced from other experiments.

FREQUENCY OF MEIOSIS WITHOUT EXCHANGE

If the conclusion that homologous strands cross at random, reached by analysis of crossing over in attached XX and in closed X^0 is applied to BRIDGES's data for the normal X chromosome (table 9), it can be shown that some crossing over between normal chromatids takes place at meiosis in all eggs in Drosophila.

TABLE 9

Percent of eggs of Drosophila melanogaster in which no exchange between chromatids of the X chromosome occurs at maturation, computed from observed crossovers from completely marked X chromosomes. (Observed values are from preliminary unpublished data of C. B. Bridges's experiment 425 C).

	NON-CROSSOVER	SINGLE	DOUBLE	TRIPLE	QUADRUPLE	TOTAL
Number	1050	1332	330	17	2	2731
Percent	38.3	48.7	12.1	0.6		
	0.6	1.8	1.8			
	37.7	46.9	10.3			
	10.3	20.6				
	27.4	26.3				
	26.3					

1.1 percent of chromatids resulting from no exchange
0.25 percent of eggs in which no exchange takes place

If exchange takes place at random among normal homologous chromatids, 8 non-crossover to 24 single crossover to 24 double crossover to 8 triple crossover chromatids is the expectation from triple exchange. The observed percent of triple crossovers from completely marked chromosomes was 0.6 percent; if three times 0.6 is subtracted from 12.1 percent of observed double crossovers there remain 10.3 percent of doubles from double exchange. The expectation from double exchange is 4 non-crossover to 8 single crossover to 4 double crossover chromatids; if twice 10.3 (which is the expected percent of single crossover chromatids due to double exchange) and three times 0.6 (the percent of single crossover chromatids expected from triple exchange) are subtracted from the observed 48.7 percent of single crossovers, there remain 26.3 percent due to single exchange. An equal percent of non-crossover chromatids is to be expected due to single exchange. When all percents of non-crossovers due to triple, double, and single exchange have been subtracted from the observed percent of non-crossovers there remain only 1.1 percent of chromatids to be expected from 0.25 percent of eggs or no eggs in which maturation has taken place without any exchange between chromatids.

Since crossing over is less frequent in heterozygotes for closed X^0 there may be some eggs in which no crossing over takes place.

The ratio of normal X non-crossovers to inviable eggs is expected by random exchange between homologous chromatids to be 1:2 as far as it is affected by single exchange, and 1:4 as far as affected by double exchange. The ratio will be increased in the proportion of 1:0 as far as no exchange occurs (tables 5 and 7). The observed ratio is 27:33, which is not inconsistent with expectation.

SISTER STRAND CROSSING OVER

Up to this point, ratios that have been examined have been based on exchange (found to be random) between homologous chromatids and it has been tacitly assumed that crossing over does not take place between sister strands. There is in fact evidence for the assumption.

STURTEVANT (from results not yet published but soon to appear) has found that crossing over does not take place between sister strands when crossing over is unequal.

The percentages of non-crossovers, crossovers, patroclinous males and inviable eggs in the population from females heterozygous for closed X^0 (table 7) are found to be consistent with expectation if the ends of the closed chromosome are inseparable, and if double or triple complexes resulting from crossing over of chromosomes are lethal, and if crossing over between non-sister chromatids is at random.

If, however, crossing over is random for all four strands the ratios depart from expectation (see table 5). Single, double, and triple exchanges between homologous chromatids would result in equal numbers of closed X^0 and normal X non-crossovers, which is almost certainly the true ratio, there being a relatively greater inviability in the closed X^0 class in stages after egg laying. If sister strands cross over, single exchange between normal sister strands would give rise to viable apparent non-crossovers, but between closed sister chromatids would give rise to an inviable double complex, reducing the percent of closed X^0 non-crossovers and also increasing the percent of inviable eggs or of patroclinous males. Double exchange if it included exchanges between sister strands would also change the ratios in the same direction. The expected ratios due to different kinds of exchange are given in table 5.

The observed ratios then show that sister strand crossing over if it occurs at all is not as frequent as crossing over between homologues. WEINSTEIN (1932) has reached the same conclusion mathematically.

Evidence that sister strand crossing over does not occur when conjugation of homologous chromosomes is prevented is derived from the experiment with heterozygous closed X^0 and inverted X (appendix, experiment J_1, table 13). Conjugation between homologous strands was almost prevented by the inversion Δ-49 and inviable complexes from single and odd numbered exchanges would have lowered the percent of the closed X^0 non-crossover class if exchange had occurred between sister closed X^0 chromatids. But no reduction was observed; the ratio of closed to normal non-crossovers was no more different from 1:1 than the deviation shown by other experiments to be probably due to differential inviability.

SUMMARY

A female Drosophila carrying attached X chromosomes and homozygous for yellow produced a daughter with a single maternal X chromosome that was "closed" (X^0).

The closed X^0 may conjugate with another X chromosome since double crossovers are recovered from females heterozygous or homozygous for closed X^0.

There is genetic evidence that the chromosome is closed by union of its ends since the loci for yellow and bobbed have never been found to be separated by crossing over, nor have the characteristics of closed X^0 ever been separated by crossing over from either of the two loci, which are near the ends of the chromosome.

No single crossovers are recovered from females carrying closed X^0 whether homozygous or heterozygous.

The percents of double crossovers among the normal offspring of females heterozygous for closed X^0 are somewhat lower than among normal controls for every region of the chromosome. The most marked difference in frequency of doubles is at the left end of the chromosome. This region in the closed chromosome is near the spindle fibre attachment, a condition which is known to decrease crossing over.

In cytological preparations the closed chromosome appears as a slightly elongated closed ring.

Low viability of closed X^0 is shown by the great difficulty in maintaining homozygous stock under the most favorable conditions, by low frequency of closed X^0 males in backcrosses, and by a high percentage of closed X^0 classes among dead pupae from backcrosses. The survival of some adults and the observations on dead pupae indicate a variable degree of inviability after the egg stage.

A single exchange of segments (crossing over at one level only) between a closed X^0 and a normal X chromatid would result in a double complex, consisting of two chromatids attached together, because the two ends of the closed chromatid are united. Among F_1s from flies heterozygous for closed X^0 there is no class of non-disjunctional females containing united X chromosomes as would be expected if double complexes survive.

A 2-strand double exchange (one in which only two homologous chromatids take part) would produce two separate double crossovers, one closed, one normal.

A 3-strand double exchange in which two normal strands and one closed strand take part would produce a normal double crossover.

The other kind of 3-strand double exchange, in which two closed chromatids and one normal chromatid take part, would unite three chromatids into one complex, and no closed X^0 double crossover would be recovered.

A nearly 1:2 ratio of the percent of closed X^0 double crossovers (among the closed X^0 F_1s from heterozygotes) to the percent of normal X doubles (among normal X F_1s) has been observed. It is the ratio to be expected if homologous strands cross at random; that is if 2-strand and each of two kinds of 3-strand double exchange are equal. These results together with the equality of 2-strand and 4-strand double exchange found by EMERSON and BEADLE (in press) in attached X show that exchange between homologous strands is at random.

Patroclinous males are about $\frac{1}{3}$ as frequent as normal X double crossovers from heterozygotes; these might result from irregularities due to formation of united chromatids at maturation and together with inviable eggs represent classes due to exchange.

Patroclinous males would be expected from the second kind of 3-strand double exchange if two united chromatids going to one pole dragged the third one that is also united to them out of its nucleus leaving one nucleus with a single X chromatid after the first division. The expected frequency of patroclinous males from 3-strand exchange would be 1/4 of the frequency of normal double crossovers, a little less than the observed 1/3. Some patroclinous males may be derived rarely from other exchanges.

The percent of double crossovers from homozygotes is in agreement with the conclusions reached from heterozygotes in regard to double crossovers. The true percent of patroclinous males from homozygotes is obscured by low viability of regular classes but the observed percent appears to be consistent with expectation on the assumption that patroclinous males are derived mainly from 3-strand double exchange.

Lethal effects of exchange would affect the egg stage of Drosophila. Inviable eggs were found to be 33.2 percent of the deduced number representing the total population in F_1 from females heterozygous for closed X^0. This is in agreement with the hypothesis that patroclinous males are derived mostly from one kind of 3-strand double exchange and that double chromatid complexes resulting from one exchange between two chromatids are lethal. On the hypothesis, patroclinous males, which are about 2.3 percent of the population, and an equal number of YO gametes, which are lethal in the egg stage, represent one kind of double crossover, and the remainder of the lethal eggs, or about 31 percent of the population, represent single crossovers. The percents are in agreement with the hypothesis if crossing over takes place somewhat less frequently between closed X^0 and normal X than between normal Xs. Evidence for reduced frequency of crossing over is found in a relatively high percent of non-crossovers among F_1s from flies heterozygous for closed X^0, and a relatively low percent of double crossovers from heterozygotes as compared with values obtained from controls.

If the first meiotic division when closed X^0 is present is reductional, nuclear division after exchanges that produce double complexes would be expected to lead to severed chromatids, but no flies containing corresponding deficiencies or duplications have been observed. Patroclinous males would be expected if some nuclei were formed without any X chromatid.

If the first meiotic division is the kind of equational division that would segregate two single chromatids from a double lethal complex the observed ratios are most nearly satisfied.

If inviable eggs and patroclinous males result from irregularities of crossing over their frequency should be reduced if crossing over is reduced. Heterozygotes for closed X^0 and normal X gave 31 percent of inviable eggs; when crossing over was reduced by an inversion in an X chromosome,

heterozygotes for closed X^0 and the inversion (Δ-49) gave only 6.3 percent of inviable eggs.

Double crossing over is almost entirely prevented and single crossing over is reduced to 13 percent in heterozygotes for normal X and inversion Δ-49. Heterozygotes for closed X^0 and Δ-49 gave 6.3 percent of inviable eggs but no excess of patroclinous males over the percent obtained from heterozygotes for Δ-49 and normal X. This agrees with the hypothesis that with closed X^0, patroclinous males represent one kind of double crossover, and inviable eggs represent almost entirely single crossovers, crossing over being relatively infrequent when closed X^0 is present.

The observed ratios are those to be expected if exchange between homologous strands is random and if sister strands do not cross over.

When conjugation between homologous strands is almost prevented by an inversion in the apposing X chromosome of a female heterozygous for closed X^0, the ratio of the two classes of non-crossovers in F_1 is 1:1 which is not expected if sister strands cross over independently of crossing over between homologues.

The conclusion that homologous strands cross at random applied to unpublished data of BRIDGES from completely marked X chromosomes, indicates that exchange between normal X chromosomes probably takes place as a rule in all eggs of *Drosophila melanogaster*.

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APPENDIX

TABLES 10–13

For every type of complementary offspring the class first recorded is that containing the left end of the maternal chromosome that is described above the line.

TABLE 10

Experiment A_1 and A_2;

♀ offspring of $\dfrac{X^0y \quad cv \quad v \quad f}{ec \quad ct^8 \quad g^2 \quad bb}$ ♀ $\times y\ ec\ cv\ ct^8\ v\ g^2\ f\ bb(X_8)\sigma^{\prime}$.

1 2 3 4 5 6 7

Experiment A_3;

offspring of $\dfrac{X^0y \quad ec \quad ct^8 \quad g^2}{sc \quad br \quad w^a \quad cv \quad v \quad f}$ ♀ $\times sc\ w^a\ ec\ cv\ ct^8\ v\ g^2\ f\sigma^{\prime}$.

1′ 1″ 1‴ 2 3 4 5 6 7

Experiment B_1;

offspring of $\dfrac{X^0y \quad cv \quad v \quad f}{sc \quad ec \quad ct^8 \quad g^2 \quad Bx^3 \quad bb}$ ♀ $\times X_8\sigma^{\prime}$.

1 2 3 4 5 6 7′ 7″

Experiment B_2;

offspring of same type of ♀ $\times y\ ec\ cv\ ct^8 v\ g^2\ f\ (X_7)\sigma^{\prime}$.

EXPERIMENT	TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED	
A_1, A_2	0	1849	2070	1,6	5	19	2,6	11	44
A_3		1687	3298		12	35		12	64
B_1		1333	1600		5	14		6	32
B_2		1371	1495		6	10		5	25
Total	0	6240	8463	1,6	28	78	2,6	34	165
A_1, A_2	1,2	2	0	1,7	3	18	2,7	10	37
A_3		1	3		4	31		14	63
B_1		0	1		2	12		5	23
B_2		0	0		1	12		8	9
Total	1,2	3	4	1,7	10	73	2,7	37	132
A_1, A_2	1,3	1	1	2,3	0	0	3,4	6	7
A_3		0	0		0	1		5	4
B_1		0	0		0	1		1	8
B_2		0	0		1	0		0	8
Total	1,3	1	1	2,3	1	2	3,4	12	27
A_1, A_2	1,4	6	8	2,4	3	13	3,5	6	18
A_3		7	9		7	18		10	34
B_1		1	6		3	11		3	16
B_2		1	2		5	8		5	8
Total	1,4	15	25	2,4	18	50	3,5	24	76

TABLE 10 (*Continued*).

EXPERIMENT	TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED	
A_1, A_2	1,5	5	20	2,5	15	36	3,6	15	48
A_a		5	16		7	43		17	44
B_1		3	14		9	13		8	28
B_2		1	13		8	12		10	27
Total	1,5	14	63	2,5	39	104	3,6	50	147
A_1, A_2	3,7	13	30	4,7	19	47	6,7	2	11
A_a		16	42		25	111		5	19
B_1		5	27		11	50		4	7
B_2		7	14		4	29		1	4
Total	3,7	41	113	4,7	59	237	6,7	12	41
A_1, A_2	4,5	6	20	5,6	5	15			
A_a		11	19		6	11			
B_1		3	12		1	8			
B_2		2	9		1	9			
Total	4,5	22	60	5,6	13	43			
A_1A_2	4,6	17	52	5,7	10	19	3,4,5,6		
A_a		14	61		11	36			
B_1		8	35		1	16			
B_2		11	31		1	13			1
Total	4,6	50	179	5,7	23	84	3,4,5,6		1

							double and quadruple crossovers		
Total	regular classes	6746	10168	non-crossovers	6240	8463		506	1705

	non-disjunctional ♀ ♀	patroclinous ♂ ♂	percent of total	percent of (X^0+patr.♂♂)
A_1A_2	5	inviable		
A_a	1	210	3.47	10.0
B_1	0	inviable		
B_2	1	86	3.0	5.6
Total	7			

TABLE 11

Control of Experiment A_1 and A_2. Female offspring of $\frac{sc \quad cv \quad v \quad f}{(sc) \; ec \quad ct^8 \quad g^2 \quad bb}$ ♀ × X_8♂. *For each type, the second class contains ec.*

2 3 4 5 6 7

TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED	
0	558	557	2,6	12	22	5,7	11	8
2	71	94	2,7	19	12	6,7	5	2
3	65	80	3,4	2	3	2,3,7		1
4	162	150	3,5	10	5	2,4,6	1	
5	107	99	3,6	14	14	2,4,7	1	1
6	104	111	3,7	11	9	2,5,7	1	2
7	63	97	4,5	5	11	3,5,7		1
2,3	2	1	4,6	22	22	4,5,7		1
2,4	4	9	4,7	17	13	4,6,7		1
2,5	15	11	5,6	1	2	3,4,5,7		1
Total non-crossover	1115		Total single and triple	1213		Total double and quadruple	295	
Grand total 2623								

TABLE 12

Experiment D_a;

♂ offspring of $\frac{X^0y \quad cv \quad v \quad f}{X^0y \; ec \quad ct^8 \quad g^2}$ ♀ × *bb Y* ♂.

1 2 3 4 5 6 7

Experiment D_b;

♂ *and* ♀ *offspring of* $\frac{X^0y \quad cv \quad v \quad f}{X^0y \; ec \quad ct^8 \quad g^2}$ ♀ × X_8♂ *or* X_7 ♂.

1 2 3 4 5 6 7

EXPERIMENT	APPARENT TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED	
D_a	0	48	46	2,3	0	0	3,6	0	0
D_b		1336	1032		1	0		7	7
Total	0	2462			1			14	
D_a	2	0	2	2,4	1	0	4,5	0	0
D_b		4	6		2	6		3	7
Total	2	12			9			10	
D_a	3	0	0	2,5	0	1	4,6	1	0
D_b		6	8		9	4		8	5
Total	3	14			14			14	

TABLE 12 (*Continued*).

EXPERIMENT	APPARENT TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED	
D_a	4	0	0	2,6	0	1	5,6	1	0
D_b		11	14		4	6		4	6
Total	4	25			11			11	
D_a	5	0	0	3,4	0	0	non-disjunc-		
D_b		10	11		4	1	tional ♀♀		2
Total	5	21			5				
D_a	6	0	0	3,5	0	0	gynandro-		
D_b		9	5		4	5	morphs	1	
Total	6	14			9				

Values deduced from apparent type 0 and apparent singles

TYPE	0	1,2	1,3	1,4	1,5	1,6	1,7	2,7	3,7	4,7	5,7	6,7
NUMBER	2457	1	1	6	8	9	5	11	13	19	13	5
Total	regular classes	2646			non-cross-overs		2457		double crossovers		189	

F_1's of females mated to X_7 males

Total	2121	Patroclinous ♂♂	254	Per-cent	12.0			

TABLE 13

Classes observed in experiments in which eggs were counted on the first day and on the fourth day after laying.

	NON-CROSSOVERS		CROSSOVERS		PATROCLINOUS ♂♂	EGGS TOTAL	EGGS INVIABLE	NON-DISJUNCTIONAL ♀♀
J_1	*y*	*cm*	*y cm*	+	*y* Δ-49 *cm bb*			+
$X^0 y/\Delta$-49 $cm^{\pm} bb$								
$\times y\,\Delta$-49 *cm bb*	2317	3157	0	0	inviable			1
first 6 days						3383	217	
J_2	*y*	g^2	$y g^2$	+	$y g^2 bb$			+
$X^0 y/g^2 \times yg\,bb$	1302	1535	136	393	135			among
first 6 days	754	849	73	224	74	3326	1040	crossovers
J_3								
g^2/Δ-49$\times g^2$								
first 6 days						3498	112	

X-RAY EFFECTS ON DROSOPHILA PSEUDO-OBSCURA

JACK SCHULTZ[1]

California Institute of Technology, Pasadena, California

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In order to procure new material for the study of problems connected with the non-disjunction of the sex chromosomes in *Drosophila pseudo-obscura*, a series of X-ray experiments was carried out. Among the species of Drosophila, *pseudo-obscura* is distinguished by the length of its X chromosome, whose genetic map is the longest recorded in any species (LANCEFIELD 1922). The work of LANCEFIELD has made a number of mutants available for experiments with this chromosome. Accordingly, the experiments were arranged to permit the detection of (1) non-disjunction of the sex chromosomes in the treated females, (2) crossover modifications in the treated X chromosomes, and (3) non-disjunction of the X chromosomes in the progeny of treated individuals. An eosin (w^e; locus 68) garnet-2 (g^2; locus 88) stock was irradiated, and the treated females were mated to wild type males. In the progeny, exceptional individuals, resulting from non-disjunction of the sex chromosomes in the mother, could be detected. The regular F_1 females were mated to males carrying in their X chromosome the dominant gene Pointed; among their offspring, the sons gave the measure of crossing over in the eosin garnet-2 interval, and the presence of the dominant sex-linked gene in the father permitted the detection of non-disjunction of the X chromosomes in the mother.

The reciprocal cross was also carried out: eosin garnet-2 males were treated and mated to wild type females. The daughters in this cross were of the same constitution as those obtained from the reciprocal mating, and were tested in the same manner. In these tests of F_1 daughters, the untreated chromosome may be considered to serve as a control. There is no control series available for the mating of irradiated female to normal male; for the reciprocal cross, however, a control was raised.

The X-ray dosage was the usual one of MULLER (1927); the Coolidge tube used at a peak voltage of 50 K.V., with a 5 m. am. current, the flies at a distance of 16 cm. from the target, with a 1 mm. aluminium filter. The exposure was sixty minutes for the females and for one set of males, but another set of males was treated for seventy-five minutes.

These experiments were carried out in the winter of 1929 and the spring of 1930, and have been reported briefly in the Carnegie Year Book for 1930 (MORGAN, BRIDGES and SCHULTZ 1930).

[1] Carnegie Institution of Washington.

THE DATA

The most striking effects were observed in the progeny of the irradiated males that were mated to normal females. There was a marked deficiency of females in the progeny from the experimental cultures, although the sex ratio in the controls was 1.16 ♀ to 1.00 ♂. This deficiency of females was most marked, as might be expected, in the series given the heaviest treatment (table 1). In that case 66 percent of the expected number of females were lacking.

TABLE 1

Progenies of treated males + ♀ ♀ × w^e g^2 ♂♂, ♂♂ exposed to X-rays; 50 K.V., 5 m. am., 1 mm. Al filter distance 16 cm.

EXPOSURE MINUTES	NUMBER OF CULTURES	♀♀	♂♂	SEX RATIO	SEX RATIO (EXP) / SEX RATIO (CONTROL)	PERCENT ♀♀ DEFICIENT
60	26	349	634	0.55	0.47	0.53
75	30	185	435	0.40	0.34	0.66
0	8	554	475	1.16	..	..

There was also a high percentage of abnormalities in the progeny; 3.5 percent of the total in the sixty minute treatment, and 2.7 percent in the seventy-five minute treatment. The two largest groups consisted of mutants of the Minute type, with the usual small bristles and other characteristic peculiarities of this class (BRIDGES and MORGAN 1923, SCHULTZ 1929), and of a number of males which had no gonads but were otherwise normal (table 2). These were very similar to the "castrated" males described by GEIGY (1927, 1931) as resulting from treatment of eggs with ultra violet light. In *Drosophila pseudo-obscura*, thea bsence of testes is particularly striking, since the envelope of the gonad is colored a bright orange, and is easily visible through the abdominal wall. In these abnormal males the abdomen appeared white. On dissection it became apparent that the testes were absent, but the rest of the reproductive system was normal.

TABLE 2

Abnormalities occurring as a result of treatment.

EXPERIMENT	TOTAL ABNORMAL IN F_1	♂♂ WITHOUT TESTES	STERILE MUTANTS	F_1 MUTANTS RETAINED	F_1 ♀♀ TESTED FERTILE CULTURES	STERILE CULTURES	VISIBLE SEX LINKED RECESSIVES	LETHALS
60 min ♂♂	35	13	10	7	46	11	6	8
75 min ♂♂	17	1	6	2	5	7	1	..
60 min ♀♀	12	..	4	..	45	23	3	4
Control	..	..	..	..	..	..	..	..

In addition to these two groups there appeared such characters as "Star" eyes, "Plexate" venation of the wings, "Spread" wings, "Confluent" wing veins, and "Delta" venation. Many of these proved sterile, but some were fertile and stocks of these were established.

The effects observed in the progeny of the treated females were much less extreme. There was in F_1 a slight deficiency of males, somewhat more than might be expected as a result of the poorer viability of the eosin garnet-2 males as compared with their wild type sisters. In the absence of a control series, the rather wide fluctuations of the sex ratio in *Drosophila pseudo-obscura* in different experiments make it difficult to place much weight on this result. The occurrence of sex linked lethals in the mother would lead to such a deficiency of males and would thus be expected in progenies from X-rayed mothers.

TABLE 3

Progenies of treated females $w^e\ g^2$ ♀♀ × + ♂♂, ♀♀ *treated as the* ♂♂ *in table 1.*

EXPOSURE (MINUTES)	NUMBER OF CULTURES	♀♀	♂♂	SEX RATIO
60	37	887	547	1.60

The number of abnormal specimens[2] was much lower in this group than in the progeny of treated males, a fact which gains significance when it is remembered that new sex-linked recessives in the treated chromosomes may be detected in this cross, which is not the case when males are treated. In spite of this, only 0.8 percent of the total progeny were variants. Of these, one was an occurrence of Notch—probably an oogonial mutation, since three such females, all of which were sterile, appeared in one culture. There occurred also a singed male (sterile), and several mosaics, which may have been due to fractional mutation[3]; a male with one wing curled, another with one miniature wing, and a third male with the venation plexus on one wing.

It is clear that the effects in F_1 are much more extreme when the males are treated. This is further evidenced in the tests of F_1 daughters. Here again (the last four columns of table 2) the males treated for sixty minutes are much harder hit than the corresponding females.

Those of the mutants that could be carried on were studied. It developed that a number of them carried translocations, indicating as might be ex-

[2] Only one male resulting from primary nondisjunction in the treated females was found.

[3] These were not tested. Their occurrence is of interest in connection with the work of PATTERSON (this journal, 1933, pp. 32–52) on *D. melanogaster*, where mosaics of this kind do not occur in the female. Among the progenies of X-rayed males, five "fractional" mutations were found among the 52 aberrants of the present experiment. These also were not tested; further data are necessary for the study of this problem.

pected, that these occur frequently following the radiation of sperm in this species. Six chromosome aberrations were found: three translocations, one involving the second and third autosomes, the others the second chromosome and the Y; a high non-disjunction stock, probably a translocation, since in such cultures sterile males, probably carrying duplications for a part of the X, appeared; and finally, two crossover reducers, one inhibiting crossing over between eosin and garnet-2, the other between yellow and eosin. The sex-linked recessives which appeared included yellow, a prune-like eye color located some twenty units to the left of yellow, an extreme short vein character associated with a total loss of the testes, an echinus-like eye, a rudimentary-like wing, and a "bubble" wing which proved allelomorphic to the similar mutant found by LANCEFIELD (1930) in race B.

DISCUSSION

The deficiency of females in progenies from X-rayed males is a phenomenon discovered by MULLER in his experiments of 1927 on *Drosophila melanogaster*. He offered the explanation that "dominant lethals" occurred in the X chromosomes as a result of irradiation, and that females heterozygous for an X chromosome carrying such a lethal died. Since the Y chromosome is genetically almost empty, the males in the progeny have an advantage over the females who receive from their fathers a possibly injurious X instead of the innocuous Y. This manifests itself in the changed sex ratio.

In MULLER'S data on *D. melanogaster*, the maximum effect found was an 18 percent deficiency of females, and this of doubtful statistical significance. The present data on *D. pseudo-obscura* show a much greater depression of the sex ratio, the deficiency of females reaching sixty-six percent of the number to be expected on the basis of the control values. It will be remembered that the X chromosome of *D. pseudo-obscura* is very long; in fact, it contains about forty-three percent of the haploid chromatin, as contrasted with twenty-eight percent in the *melanogaster* X chromsome. The more extreme results with *D. pseudo-obscura* are then to be expected on MULLER'S hypothesis, since the longer the X chromosome, the greater the chance that a "dominant lethal" will arise, hence the greater the depression of the sex ratio. The problem is complicated, however, by such factors as the comparative extent of the "inert region" of the X in the two species, and the relative importance of different regions and different chromosomes in the production of dominant lethals. It is entirely likely that these represent long deficiencies, or translocations resulting in chromosomes with two spindle fibres, which would be lethal.

In this connection, MULLER'S attempt to test the assumption that "dominant lethals" occur at random throughout the chromosomes may be

mentioned. On this assumption he deduced a relation between the depression of the sex ratio, the percentage of eggs laid that hatched into adult females and the proportion of the total haploid chromatin contained by the X chromosome. He found this relation not to hold in his data, nor does it hold in HANSON's (1928) data even when correction is made for the "inert" region (PAINTER 1931, MULLER and PAINTER 1932, DOBZHANSKY 1932) of the X. Many more eggs die than should according to formula. MULLER therefore considered that some type of effect on more than one chromosome might be responsible. The explanation of the discrepancy, offered by EFROIMSON (1931), that an autosomal deficiency of a given length is more likely to be a dominant lethal than one in the X chromosome, on considerations of genic balance, seems unlikely. For example, a relatively small section of the X is involved in PATTERSON's "viability gene" (1932) which behaves as a dominant lethal under his experimental conditions, although BURKART (1931) appears to have obtained from the "Blond" translocation a deficiency for this section which survives as a Minute. Furthermore deficiencies in the autosomes, involving several units of the map, are known to be viable. It seems preferable to adopt the view that the many possible types of multiple chromosome aberrations resulting from X radiation are responsible for the excess mortality.

The depression of the sex ratio seems to be the easiest way of measuring the gross X-ray effects in those experiments where the interest is centered on the magnitude of the effect rather than on the detailed analysis of the various types of disturbance. In this respect, *D. pseudo-obscura* is an especially favorable object due to the extent of the effect. Yet by matings of the treated males to attached X females in *D. melanogaster*, a converse deficiency of males may be obtained (BARTH 1928); this is, of course, due to the dominant lethals plus the sex linked recessive lethals. These two quantities combined give a discrepancy of sufficient magnitude to be workable in *D. melanogaster*. The technique has the advantage of being less laborious than the usual ClB method of MULLER, and therefore may have a certain use in the further analysis of the effects of X-rays on chromosomes, and particularly in exploratory work with other agents.

It may be remarked in the present work, as in that with *D. melanogaster*, that the X-ray effect is noticeably less upon the female than upon the male (MULLER 1930, PATTERSON and MULLER 1931). In *D. melanogaster* this has also been shown to be the case for the immature germ-cells of the male as compared with the mature sperm (HARRIS 1929, HANSON 1929) suggesting that the difference in effect may be perhaps correlated with the difference between haploid and diploid. It has been indicated that the difference between the effect on male and female in *D. melanogaster* is due largely to a difference in the number of chromosome abnormalities (PAT-

TERSON and MULLER 1931). It is possible that the usual number of these abnormalities may be produced in the diploid cell, but that they are eliminated because a greater proportion of types, which cannot undergo division normally, are produced. It should also be remembered however, that the relative distances between chromosomes are greater in the immature cells than they are in the sperm, hence translocation might less frequently be successful. When these considerations are taken into account, the observed differences are perhaps to be expected.

The males without gonads found in F_1 from the treated males deserve special mention. Their condition is not due to loss of the Y chromosome, since XO males of this species are normal in testis size. The lack of testes must then be either a developmental abnormality caused by X-rays, or a rather frequent kind of dominant mutation, affecting either the initial establishment of the germ track, or the later development of the gonad. It should be recalled here that among the mutants induced by X-rays, one sex-linked recessive caused a loss of the testes, associated with a short vein character in the wings. The male sterility observed by MULLER in his experiments with *melanogaster* may be due to the same phenomenon, which is less easily observed in *D. melanogaster*, since the color of the testis envelope is relatively inconspicuous.

An accurate comparison of the effects of X-rays on *D. melanogaster* and *D. pseudo-obscura* cannot be made at present since no strictly comparable data are available. The comparison might be interesting, in view of the results of TIMOFÉEFF-RESSOVSKY (1931) on *D. funebris*, where the data, also unfortunately not strictly comparable, indicate a lower lethal mutation rate than in *melanogaster*. In *D. pseudo-obscura* it is the impression that dominant mutations are more frequent than in *D. melanogaster*, both spontaneous mutations and those from X-rays.

SUMMARY

1. X-ray experiments were made with *Drosophila pseudo-obscura*, which were so arranged that non-disjunction could be detected in the treated females, and modifications of crossing over and disjunction of the X chromosomes could be detected in the F_1 both from treated males and females.

2. In the progeny of treated males, instead of the sex ratio of 1.16 ♀ : 1.00 ♂ observed in the controls, there occurred a marked decrease from the expected number of females. Further a high percentage of abnormalities was found. They consisted mostly of Minutes and males without gonads, and a number of other mutants in addition. Six chromosome aberrations were found as a result of treatment.

3. As has been found in the work with *D. melanogaster*, the effects noted in the progeny from treated females were less extreme than in those from treated males.

4. The more extensive depression of the sex ratio observed in *D. pseudo-obscura* than in *D. melanogaster*, in the progenies from treated males, may be correlated with the difference between the relative sizes of X and autosomes in the two species. The longer the X relative to the autosomes, the greater the chance of injury to the females carrying such chromosomes that have been irradiated.

5. The depression of the sex ratio in *D. pseudo-obscura* affords the simplest technique available for measuring gross X-ray effects on chromosomes. A similar technique in *D. melanogaster* is available from the mating of treated males to attached X females.

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